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JAMES M. VAN HOOK (1870–1935)

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James M. Van Hook, Professor of Botany in Indiana University, died at Pentwater, Michigan, June 20, 1935, after an illness of two years. His remains rest in the cemetery at Pentwater, Michigan. Survivors include the widow; a son; a daughter; a brother; and a sister.

Professor Van Hook was born in Washington County, Indiana, on December 19, 1870. For this early education Professor Van Hook attended the public schools with graduation from the Borden Institute of Borden, Indiana, in 1894. He attended Indiana University, where he received the A.B. degree in 1899, and the A.M. degree in 1900. He then entered Cornell University as a graduate student and assistant in botany, for a period of one year, and from 1902–04 he was assistant in plant pathology in the extension department of Cornell University. During his graduate work at Cornell he studied under Professor G. F. Atkinson. From 1904 until 1907 he was assistant botanist, Ohio Agricultural Experiment Station, with principal work in plant pathology, and was author of a number of station bulletins dealing with plant diseases. During his graduate work at Cornell, and later, at the Ohio Agricultural Experiment Station, much of his research was devoted to the disease of ginseng. The ginseng plant was, at that time, being grown extensively under cultivation, and the growers were confronted with a number of ginseng diseases, which were not well known.

That this young investigator, primarily a mycologist, could turn all the resources of his training, his knowledge, and his natural ingenuity to the practical problems of the control of plant diseases, was well demonstrated in his work on the diseases of ginseng. His findings were an important contribution to our knowledge of the diseases of ginseng and their control, and still stand as significant in its field.

In 1907 Professor Van Hook returned to Indiana University as assistant professor of botany. He was promoted through the various ranks, receiving the title of professor in 1925. At the time of his death he had thus completed almost three decades of service in that university.

During this period of service there were many students to teach, and a large part of this load fell to him. His teaching was characterized by an enthusiasm for his work, and an ability to impart this enthusiasm to the

interested student. He was acquainted with related fields as well as with his specific subject; and was able to present the facts and relationships clearly and concisely. He unselfishly shared his time and energy in and out of the classroom and laboratory, in personal sympathy and instruction. This seemed to him more important than fine-drawn definitions, and excessive theory in teaching. I am not aware that Professor Van Hook ever took a formal course in education, but I am certain he possessed the qualities of a highly successful teacher. His broad experiences and thorough training in mycology and plant pathology were brought to play in his teaching. That his ability was recognized and appreciated, was evidenced in the number of graduate students working under him for advanced degrees.

During his twenty-eight years of teaching at Indiana University his research was for the most part in mycology, with an occasional problem of diseases caused by fungi. His special field of study was with the Fungi Imperfecti. Unfortunately much of his work on this group remains unpublished. Prof. Van Hook, for the past 25 years, had been making a survey of the fungus flora of the State of Indiana, and, excepting the Uredinales, he contributed more to the knowledge of the fungi of Indiana than any other worker. The results of this survey have been published in part in the Proceedings of the Indiana Academy of Science.

In his work Professor Van Hook was opposed to the making of new species but believed where possible, a greater service could be rendered botanical science by a careful study of material collected in quantity and in various stages of development, and by appending corrections, and additions to already given meager descriptions. His extreme conservatism in naming new species is evidenced in the amount of his unpublished work on new species of imperfect fungi. His work on parasitic fungi was greatly enhanced by his familiarity with the taxonomy of the native host plants; he also was a specialist on ornamental plants.

By natural inclination, Professor Van Hook was of a quiet and retiring disposition. I believe few realize the extent and variety and thoroughness of his attainments. As a foundation he was well schooled in the classical languages and literatures and in music. He was a skilled craftsman. He brought these accomplishments to his service as a scientist. And through all he was a gentleman and a man of character.

His friendships were deep and lasting. Those who knew him recognized and admired his integrity, sincerity, kindness, and loyalty, and were impressed with his knowledge, skill, and ability in the field of botanical science, which he loved.

Professor Van Hook's interest in everything life presents made him a valuable member of his home and university community; made him an

enthusiastic cooperator in all efforts to make life more beautiful in every way and more enduringly satisfying.

Professor Van Hook was a fellow of the American Association for the Advancement of Science and of the Indiana Academy of Science; charter member of the American Phytopathological Society and of the Mycological Society of America; member of the Crop Protection Institute, the Botanical Society of America, Phi Beta Kappa and Sigma Xi.

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THE RELATION OF APHIDS TO THE TRANSMISSION OF BEAN MOSAIC[1]

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INTRODUCTION

In practically all of the bean-growing regions of the United States, bean mosaic is a major factor in yield reduction, especially in some of the principal varieties. Although many species of insects have been mentioned as vectors of bean mosaic, no systematic search has been made of those feeding on beans in relation to the secondary spread of the virus. Bean mosaic is seed-borne, and it is through diseased seed that centers of infection are established. Investigations (6, 8) have shown that primarily infected plants seldom produce more than 50 per cent infected seeds, and secondarily infected plants even less. Seed transmission, although of extreme importance, fails to explain the rapid and widespread dissemination of mosaic in some regions during certain seasons. Fields planted with reasonably healthy seed stock often contain very high percentages of the disease at the end of the season.

This paper presents data on the prevalence of aphids in bean fields in relation to the spread of mosaic, together with data on the various species of aphids found on beans. Virus-transmission studies with 12 species of aphids, as well as with 10 other species of insects commonly collected in bean fields, also are described.

REVIEW OF LITERATURE

Nelson (5) was the first to report the transfer of bean mosaic by an insect. He succeeded in transferring the virus by *Macrosiphum solanifolii* Ashm., but was unable to transmit it with the bean leaf hopper, *Empoasca fabae* (Harris). Elmer (2) believed that the mealy bug, *Pseudococcus maritimus* Ehrh., was a vector and Fajardo (3) reported positive transmission of the mosaic virus by means of *Aphis rumicis* L., *Myzus persicae* Sulz., *Macrosiphum solanifolii*, and an undetermined species of mealy bug. He

[1] The writers gratefully acknowledge the assistance given by P. W. Mason of the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, for determining several species of aphids reported in this paper. They also are indebted to C. F. Andrus for assistance in the collection of the data taken at Arlington, Virginia, and to L. L. Harter for advice and aid during the progress of the investigation and for assistance in the preparation of the manuscript.

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failed to transmit the virus by means of leaf hoppers, 12-spotted and striped cucumber beetles, red spiders, thrips, the capsid bug *Lygus pratensis* L., and a white fly, *Aleyrodes* sp. Pierce and Hungerford (8) transmitted the bean virus with an undetermined species of black aphid but not with a species of green leaf hopper, green aphid from alfalfa, and the tarnished plant bug. Merkel (4), who studied the mosaic diseases in the Papilionaceae, reports that *Aphis rumicis* and *Illinoia pisi* transmitted the viruses of *Phaseolus vulgaris* L., *Pisum sativum* L., *Lathyrus odoratus* L., *Lupinus luteus* L., *Melilotus altissima* Thu., *Trifolium hybridum* L., *T. repens* L., *Anthyllis vulneraria* L., and *Vicia faba* L. The results led the author to the conclusion that the same virus was responsible for all of the apparently different mosaics. Smith and Barker (9), in Haiti, report the transmission of bean yellows, resembling mosaic, by *Empoasca* sp. Zaumeyer (10) reported bean-mosaic transmission with *Macrosiphum solanifolii, Aphis rumicis, Myzus persicae, Illinoia pisi* Kalt., *Aphis gossypii* Glov., *Brevicoryne brassicae* L., *Hyalopterus atriplicis* L., and *Macrosiphum ambrosiae* Thos. Pierce (7) secured transmission with bean mosaic, as well as with a new virus known as yellow bean mosaic with both the pea aphid, *Illinoia pisi*, and the potato aphid, *Macrosiphum solanifolii*.

METHODS

The studies on the spread of mosaic in the field and also on the aphid populations found on beans were carried on both at Greeley, Colorado, and at Rosslyn, Virginia. In Colorado, 25 fields of the Stringless Green Refugee variety were chosen in which 2 plots, each containing 100 plants, were staked off in order to afford a means of checking weekly the presence of aphids and the development and spread of mosaic. Likewise, 100 plants were chosen at random every week in each field and the mosaic spread determined. In Virginia the data were collected in the experimental plots of the United States Department of Agriculture.

The transmission studies were carried out under cages in a greenhouse with temperature maintained at about 75–85° F. The aphids were collected in the field, where they were found colonizing on some particular host, or reared in the greenhouse. The following aphids were reared from single colonies under cages: *Aphis gossypii, Illinoia solanifolii, I. pisi*, and *Myzus persicae*. All of the other species used in the transmission experiments were collected from various host plants, as shown in table 3. These collections usually were made from individual plants within a species where the aphids were found colonizing in large numbers. In cases where it was believed a mixture of species occurred, the aphids were not used. Where the same species of aphid was found colonizing on several different host plants, as for example *A. rumicis*, which was collected from 6 different hosts, the aphids

were always identified from each host plant. All individual aphids were not identified, however a random sample of each collection was used for identification purposes. In no case was a mixture of species reported.

Aphids were then transferred to mosaic-infected Stringless Green Refugee seedlings, either by means of a camel-hair brush, or by removing portions of the plant upon which the aphids were abundant, and then later placing them on the leaves of the infected seedlings. The aphids migrated to the leaves of the bean plants and were allowed to feed for about 24 hours on the diseased ones. They were then retransferred to healthy seedlings of the Stringless Green Refugee variety by either of the two methods mentioned above. The pubescence of the dorsal side of bean leaves makes it difficult to transfer aphids by means of a camel-hair brush. However, by removing portions of the leaf upon which the aphids were feeding and placing them upon the healthy plant, little difficulty was encountered in the natural migration of the aphids. Since bean mosaic is not readily transferred except by rubbing healthy leaves with the expressed virus juice, there was no danger of transferring the virus if the leaves were carefully handled. The transfers were made under cages and the plants were allowed to remain there until mosaic symptoms appeared. Healthy check plants were placed adjacent to the cages in the open greenhouse.

Thrips, feeding on mosaic-infected bean plants, were collected in the field and transferred to healthy plants in cages by the same method described for aphids.

Leaf hoppers and other insects with sucking and chewing mouthparts were collected in the field by means of an insect sweeping net and placed in insect transmission vials. These vials containing 5 to 9 insects each were clipped on the foliage of diseased plants for a period of 1 to 8 days, and then the insects were transferred to healthy bean seedlings. Suitable check plants always were placed outside the cage.

PREVALENCE OF APHIDS IN BEAN FIELDS AND THE SPREAD OF MOSAIC

During the early part of the investigations, it was noted that very few aphids were caught by insect sweeps made in bean fields. Only after a careful examination was made of the underside of the leaves were aphids found to frequent beans in any numbers.

A careful examination of plants in the field indicates that beans apparently are not a favored host of aphids. Although many different species may be found on them, especially during the early part of the growing season, the writers have never found them in large numbers. The greatest infestation usually occurs about ten days after the beans are above ground. In Colorado they sometimes have averaged as many as 5 to the plant, but usually not more than 1 or 2. In the experimental plots at Rosslyn, Vir-

ginia, as many as 37 aphids were found on a single plant. As the season progresses, the populations decrease and, when the beans are mature, it is often difficult to find any.

It is believed that the aphids are transitory on beans and that they disappear when more favored hosts are found. Beans may serve only as a place for the aphids to obtain nourishment in their migrations. The writers have made numerous tests to determine whether aphids will colonize on beans and have found that if they are allowed to remain on beans from 48 to 72 hours they usually die. It is probable that the aphids do not long remain on a single plant, but in their search for a preferred host may visit many plants in a day. In doing this they may insert their mouthparts into the leaf and, finding it unfavorable, migrate to other plants.

Although no tests have been made to determine how long aphids must feed on a mosaic infected plant to become infective, it is believed that they acquire the virus on their first feeding and are able to infect healthy plants immediately. If the incubation period were longer it is believed little transmission of the mosaic would take place since, as pointed out, aphids cannot live on beans for any length of time.

SPECIES OF APHIDS FOUND ON BEANS IN THE FIELD

Although aphids are not found in large numbers nor throughout the entire growing season, the following species have been collected from beans: *Hyalopterus atriplicis* L., *Aphis gossypii* Glov., *Aphis medicaginis* Koch, *Aphis rumicis* L., *Illinoia pisi* Kalt., *I. solanifolii* Ashm., *Myzus persicae* Sulz., *Brevicoryne brassicae* L., *Myzocallis ononidis* Kalt., *Monellia* sp., *Calaphis* sp., *Aspidaphis* sp., *Macrosiphum* sp., and *Tritogenaphis* sp. A careful search would probably reveal many other species.

In 1931, at Rosslyn, Virginia, a collection was made of the various species of aphids on beans. The potato aphids, *Illinoia solanifolii*, accounted for 60 per cent, the bean aphis, *Aphis rumicis*, for about 20 per cent, the pea aphid *I. pisi*, for 10 per cent, the peach aphis, *Myzus persicae*, for 5 per cent, and the remaining species for 5 per cent of the aphids collected. It is quite probable that the relative percentages of the different species depend to a large extent on the crop growing in close proximity to bean fields. In Colorado, *Hyalopterus atriplicis*, *A. medicaginis*, and *A. rumicis* were found most commonly on beans. *Hyalopterus atriplicis* feeds to a large extent on *Chenopodium album* L., a common weed found in the cultivated fields of northern Colorado. *A. medicaginis* is a general feeder, but is found feeding largely on legumes such as alfalfa, *Medicago sativum* L., sweetclover, *Melilotus alba* Desr., and yellow sweetclover, *Melilotus officinalis* L. These hosts grow both under cultivation and wild along fence rows and irrigation ditches. *A. rumicis* is found to a great extent feeding on beet, *Beta vulgaris* L., an important crop plant in Colorado.

TRANSMISSION OF BEAN MOSAIC BY APHIDS

Although a few species of aphids were shown by other investigators (3, 4, 5, 6, 8) to be able to transmit the bean-mosaic virus, they never have been found numerous enough in bean fields to be suspected as primary vectors of the disease. Since many species were found feeding on beans in the field, tests were made to determine whether a number of different species would transfer the virus from diseased to healthy plants.

Owing to insufficient numbers, it was not possible in every case to use in these studies all of the species found on beans. Of the 14 species found on beans, 6 were not collected in large enough numbers for testing. They are as follows: *Myzocallis ononidis*, *Monellia* sp., *Calaphis* sp., *Asphidaphis* sp., *Macrosiphum* sp., and *Tritogenaphis* sp. Furthermore, 4 other species, not found on beans, also were used. Since aphids in general do not colonize on beans in any appreciable numbers, the 12 species used were collected in sufficient numbers from various hosts, where they were readily found.

The aphids used in the transmission studies were collected from 17 hosts as follows: *Aphis gossypii* from *Cucumis* sp., *Aphis medicaginis* from *Amaranthus retroflexus* L. and *Phaseolus lunatus* L., *Aphis rumicis* from *Trifolium repens* L., *Papaver omniferum* L., *Dahlia* sp., *Nasturtium* sp., *Rumex crispus* L., and *Cosmos* sp., *Aphis spiraecola* from *Spirea vanhouttei* Zabel, *Brevicoryne brassicae* from *Nasturtium* sp., *Hyalopterus atriplicis* from *Chenopodium album*, *Rhopalosiphum pseudobrassicae* Davis from *Brassica rapa* L., *Macrosiphum ambrosiae* Thos. from *Aster* sp., *Illinoia solanifolii* from *Solanum tuberosum* L., *Illinoia pisi* from *Pisum sativum* L., *Myzus persicae* from *Amaranthus retroflexus* L., and *Neothomasia populicola* Thos. from *Populus deltoides* Marsh. Table 1 shows the results of bean mosaic transmission with the above named species, under greenhouse conditions.

It is apparent from table 1 that all of the aphid species studied can transmit bean-mosaic virus from diseased to healthy plants, with the exception of *Neothomasia populicola*, collected from cottonwood. As previously pointed out, not all of the species used were collected from beans; but it is likely that many of them would be found on the host if extended searches were made. Since ability to transmit the virus is not necessarily confined to one species of aphid, it is likely that numerous other species not tested also would be able to spread the virus.

PREVALENCE OF APHIDS IN RELATION TO THE SPREAD OF MOSAIC

Since bean mosaic is seed-borne, the infective principle is available to the aphids as soon as the plants appear above ground and it is at this time that aphids are most numerous on beans. A short time after aphids feed

TABLE 1.—*Results of cage experiments on aphid transmission of bean mosaic*[a]

Species of Aphid	Host	Plants inoculated	Plants diseased	Infection	Check	Checks diseased
				Per cent		
Aphis gossypii	Cucumis sp.[b]	62	38	61	12	0
Aphis medicaginis	Amaranthus retroflexus[b] Phaseolus lunatus[b]	27	12	44	12	0
Aphis rumicis	Trifolium repens[b] Papaver somniferum[b] Dahlia sp.[b] Nasturtium sp.[c] Cosmos sp.[b] Rumex crispus[c]	110	79	72	12	0
Aphis spiraecola	Spirea vanhouttei[b]	30	26	87	12	0
Brevicoryne brassicae	Nasturtium sp.[b]	14	8	57	12	0
Hyalopterus atriplicis	Chenopodium album[b]	15	14	93	12	0
Rhopalosiphum pseudobrassicae	Brassica rapa[b]	10	10	100	12	0
Macrosiphum ambrosiae	Aster sp.[b]	36	28	79	12	0
Illinoia solanifolii	Solanum tuberosum[c]	83	48	59	12	0
Illinoia pisi	Pisum sativum[c]	50	26	52	12	0
Myzus persicae	Amaranthus retroflexus[c]	28	23	82	12	0
Neothomasia populicola	Populus deltoides[b]	19	0	0	12	0
	Average per cent infection			64		
	Total	484	312		144	0

[a] The Stringless Green Refugee variety was used in all experiments.

[b] Aphids from these hosts identified by P. W. Mason, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

[c] Aphids from these hosts identified by W. J. Zaumeyer.

on the plants they become a source of infection. The secondary spread of mosaic in fields containing high percentages of seed-borne mosaic is usually very rapid, while in fields containing a small amount, the disease may not increase. There apparently is a direct correlation between the size of aphid populations and the amount of mosaic spread. However, a small population in fields containing a high percentage of seed-borne infected mosaic plants may spread the disease more rapidly than a large population of aphids in a field containing a small percentage of seed-borne mosaic.

Table 2 shows data collected at Greeley, Colorado, and Rosslyn, Virginia, on the spread of bean mosaic in relation to the prevalence of aphids throughout most of the growing season. At Greeley the data on the percentage of mosaic and the number of aphids per plant were based on the

TABLE 2.—*Data showing mosaic spread and the prevalence of aphids on beans*[a]

Location	Date	Number of plants	Percentage of mosaic	Per cent weekly increase	Ratio of aphids to plants
Greeley, Colo., 1930[b]	6/20	300	7.0	.....	1:1
	6/27–30	300	15.7	8.7	1:1
	7/3–7	300	19.4	3.7	1:2
	7/12–14	300	30.3	10.9	1:7
	7/19–22	300	38.9	8.6	1:14
	7/26–28	300	41.3	2.4	Few
	8/1–4	300	41.8	0.5	None
Total mosaic increase				35.6	
Rosslyn, Va., 1931	6/15	320	.....	.....	5:1
	6/18	320	.....	.....	2:3
	6/22	320	13.0	.....	1:1
	6/29	320	50.0	37.0	1:1
	7/6	320	72.4	22.4	1:5
	7/13	320	97.0	24.6	1:2
	7/20	320	99.0	2.0	1:40
Total mosaic increase				86.0	

[a] Stringless Green Refugee variety.

[b] Data based on average of 300 plants in each of 25 fields.

average of 300 plants in each of 25 fields. At Rosslyn, the data were obtained from 320 plants in one plot.

It is evident from table 2 that, although the aphid population on beans was relatively small at Greeley in 1930, and at Rosslyn in 1931, the spread of mosaic was considerable. The difference between Greeley and Rosslyn in regard to the percentage increase is unquestionably due to the difference in the prevalence of aphids, although it is probable that there was more seed-borne infection in the beans grown in Virginia than in those grown in Colorado.

As the season progressed the aphid populations decreased. In Colorado, on June 20, the aphids averaged 1 per plant and from August 1–4 no aphids were found. A similar decrease occurred at Rosslyn, Virginia. On June 15, the aphids averaged 5 per plant and on July 20, the last date observations were made, there was an average of 1 to 40 plants.

Under field conditions the mosaic symptoms are noticeable about 10 to 12 days after inoculation, although the plant may act as a source of infection long before this. From table 2 it can be seen that, in Colorado, mosaic increased 8.7 per cent from June 20 to June 27–30, whereas during the following week the increase was only 3.7 per cent.

The aphids feeding during the week preceding June 20 are probably responsible for the spread of mosaic reported on June 27–30. Those feeding on beans from June 20 to June 27–30 account for the mosaic for July 3–7. Since the average number of aphids found on June 20 and for the following week were approximately the same, it would be assumed that the mosaic increase on July 3–7 should be equally as great as, if not greater than, for the week of June 27–30.

This difference in the actual mosaic spread and the expected spread of the disease may be accounted for in one of two ways. On June 20, when the first mosaic reading was made in Colorado, the beans were in the primary-leaf stage, at which time mosaic symptoms are difficult to diagnose. It is probable that, because of this, some infected plants were overlooked and actually a higher percentage of mosaic was present on June 20 than the amount recorded. If this were true, the percentage of mosaic increase for the week of June 27–30 would have been less than 8.7 per cent. Likewise, the aphid population may have been greater during the week preceding June 20 than those recorded for this date. This could have accounted for a considerable spread of mosaic appearing about June 27–30 and a decrease the following week because of a reduced aphid population.

The greatest mosaic increase in Colorado was noted from July 12–14 about 10 days after a relatively large aphid population, together with a considerable amount of mosaic in the field. The weekly spread of mosaic and aphid population continued to decrease from this date until the experiment

TABLE 3.—*Data showing spread of mosaic in relation to prevalence of aphids and seed-borne infection*[a]

Date	Field No. 1		Field No. 2		Field No. 3		Field No. 4	
	Per cent mosaic	Ratio of aphids to plants	Per cent mosaic	Ratio of aphids to plants	Per cent mosaic	Ratio of aphids to plants	Per cent mosaic	Ratio of aphids to plants
6/21	0	1: 6	0.3	1: 3	0.3	1: 2	0	2: 3
6/30	0.6	1: 6	0.3	2: 1	0.3	1: 1	2.0	1: 3
7/7	1.3	1: 10	0.5	1: 6	1.3	1: 3	2.3	1: 3
7/14	2.0	None	4.3	1: 6	6.0	1: 4	9.0	1: 6
7/18	4.7	"	11.3	1: 10	12.0	1: 5	20.0	1: 6
7/25	7.0	"	12.0	None	17.3	1: 8	30.0	1: 8
8/1	8.0	"	13.0	"	19.7	None	31.3	None
Total mosaic increase	8.0		12.7		19.4		29.3	

[a] The data recorded in each field are based on 300 plants of the Stringless Green Refugee variety.

TABLE 3—(*Continued*)

Date	Field No. 5		Field No. 6		Field No. 7		Field No. 8	
	Per cent mosaic	Ratio of aphids to plants	Per cent mosaic	Ratio of aphids to plants	Per cent mosaic	Ratio of aphids to plants	Per cent mosaic	Ratio of aphids to plants
6/21	1.5	1:3	14.5	1:2	13.0	1:1	4.0	2:1
6/30	12.0	1:4	35.0	1:8	26.3	1:2	20.0	1:1
7/7	12.3	1:8	36.7	1:4	36.0	1:3	42.0	2:3
7/14	17.3	1:10	47.0	1:8	46.0	1:6	67.7	1:3
7/18	25.0	None	57.3	1:10	51.3	1:6	72.7	1:6
7/25	28.7	"	58.7	None	52.3	None	73.0	1:10
8/1	29.0	"	60.3	"	59.7	"	73.0	None
Total mosaic increase	27.5		46.8		47.3		69.0	

was terminated. Because of the increased number of infected plants, fewer aphids are required to transmit a reasonably high percentage of mosaic later in the season, while, at the same time, the decrease in number of aphids apparently accounted for the decrease in secondary spread.

The data obtained at Rosslyn, Virginia, while showing a greater secondary spread of mosaic, are similar to those taken in Colorado. Mosaic symptoms were first observed on June 22, and increased a week later by 37 per cent. This was due possibly to the large aphid population about 2 weeks previously, which averaged 5 to the plant. Since there was considerable initial infection, the spread was rapid. The number of aphids decreased, later accompanied by a decrease in the secondary spread.

This decrease in secondary spread of mosaic late in the season, especially in fields containing a high percentage of infection, may not have been entirely due to a decrease in number of aphids. Since aphids presumably feed at random, the chances of their feeding on healthy plants after becoming infective would necessarily decrease as the proportion of mosaic plants increased. It is apparently for this reason as well as for a decrease in number of aphids that the secondary spread of mosaic does not increase so rapidly late in the season as it does earlier.

The secondary spread of mosaic is dependent not only on the number of aphids present but also on the amount of seed-borne infection present early in the season. In this connection, 8 bean fields were chosen to study the relationship of mosaic spread to aphid prevalence and seed-borne mosaic infection. The amount of mosaic in these fields at the beginning of the study ranged from 0 to 14.5 per cent and the aphid populations from relatively small to reasonably larger. The results of the observations are recorded in table 3.

In comparing the mosaic increase in field No. 1 with that of field No. 4, it can be seen that the spread in the latter is almost 4 times as great as in the former. In both fields no mosaic was evident on June 21. The aphid populations in field No. 1 were less throughout the season than in field No. 4, which accounted for this difference in the spread of mosaic. In fields No. 2 and No. 3, where the amount of mosaic infection was only 0.3 per cent on June 21, the mosaic had increased to 12.7 per cent in field No. 2 and to 19.4 per cent in field No. 3. Although the aphids were more numerous in the former field on June 30 than in the latter, more were present in field No. 3 than in field No. 2 throughout the entire season.

A comparison of field No. 5 with No. 8 shows that although the initial amount of mosaic infection was slightly greater in the latter than in the former, the amount of secondary spread was much greater in field No. 8 than in No. 5. This can be explained by the larger aphid population in field No. 8 than in field No. 5. In field No. 5 the largest population of aphids aver-

aged 1 for every 3 plants on June 20. After July 22 no aphids were present. On the other hand, in field No. 8 the aphids averaged 2 to the plant in the early part of the season and some were present as late as July 25.

The plants in fields No. 6 and No. 7 had approximately the same amount of initial infection on June 20 and about the same amount of secondary spread at the time when the data were completed. The aphid populations in both fields varied slightly, but, in general, they were approximately the same. Field No. 6, which had a greater amount of primary infection and fewer aphids than field No. 7, showed less secondary mosaic spread.

The evidence brought out in the eight fields, table 3, shows that with a small amount of seed-borne mosaic, present early in the season, a reasonably small population of aphids spread mosaic less rapidly than a larger population. On the other hand, a small population of aphids present in a field with a considerable amount of primary mosaic will spread very rapidly. A large aphid population in a field with a small amount of mosaic in the early part of the season produces considerable spread of the disease. This is shown in field No. 8, where, on June 20, there was 4 per cent mosaic with a population of two aphids per plant. Nine days following this the mosaic had spread to 20 per cent and by August 1, there was 73 per cent mosaic.

The writers and previous investigators have observed that mosaic is usually more severe along the borders of the field than elsewhere. In numerous instances as much as 15–20 per cent infection has been noted in the outermost rows, where only a trace was observed in the central portion of the field. This point was clearly demonstrated in two particular instances where detailed records were made on the weekly spread of mosaic in the Stringless Green Refugee variety. Two plots of 100 plants each were staked off in two fields and mosaic counts were made each week. In field No. 1 the first plot was located adjacent to an alfalfa field, while the second was near the center of the field. In field No. 2 the first plot was planted close to a field of rye seeded to alfalfa, while the second was also near the middle of the field. In both fields, plot No. 1 showed considerably more secondary spread than the plots located in the center of the bean field. In plots No. 1 and No. 2 of field No. 1 there were 3 per cent and 2 per cent of mosaic, respectively, on June 21 when the first mosaic reading was made. On August 1, when the experiment was terminated, the beans in plot No. 1 had 41 per cent mosaic, while in plot No. 2 the disease had increased to 18 per cent. The mosaic in plot No. 1 of field No. 2 had increased from 0.5 per cent to 24 per cent from June 21 to August 1, while the mosaic in plot No. 2, located in the center of the field, had increased only 7 per cent during the same time. The increases of mosaic spread of 23 per cent between the two plots in field No. 1 and of 17 per cent in field No. 2 are undoubtedly accounted for by the presence of larger numbers of aphids migrating from alfalfa and rye fields to the beans in the border rows.

Although no detailed records have been made to correlate the insect populations found in border crops and weeds with those found in the outermost rows and in the center of bean fields, observations indicate that early in the growing season more insects occur in the outermost rows than elsewhere in the field. Later in the season the insects seem to be numerous in all portions of the field. Regarding the prevalence of aphids, it appears that the crop growing adjacent to a bean field is in general correlated with the number and species of aphids found in bean fields. It is likely that larger populations of aphids would be found on beans, especially if they were planted adjacent to a leguminous crop such as alfalfa or sweetclover. Since these crops appear early in the spring they afford an ideal feeding and breeding place for aphids. They may migrate later to other near-by crops. Since it has been shown (11) that the virus diseases of a number of legumes are transmissible to beans producing symptoms that are often difficult to distinguish from the common bean mosaic, the increased severity of mosaic along the field borders may be accounted for in two ways; either by the transmission of the virus from these legumes to beans by aphids, or by the spreading of the common bean mosaic from one plant to another.

Prevalence of Other Insects on Beans[4]

A systematic search was made for other insects on beans in Colorado, to determine if some of them might also be responsible for the secondary transmission of the virus. Some years the flea beetles *Epitrix cucumeris* Harris, and *E. fuscula* Crotch, the Mexican bean beetle, *Epilachna corrupta* Muls., and the grasshopper *Melanoplus* sp. are very numerous on beans, often causing severe damage in certain sections.

Many other species of insects were observed on beans, a few of the more common ones being: The clover leaf hopper, *Aceratagallia sanguinolenta* (Prov.), the bean leaf hopper, *Empoasca fabae* (Harris), *E. filamenta* DeL., the tarnished plant bug, *Lygus pratensis* L., *L. elysius* Van D., the false chinch bug, *Nysius ericae* Schilling, the striped cucumber beetle, *Diabrotica vittata* Fab., *Thrips* sp., and many others.

Transmission studies were conducted with all of these insects, except the Mexican bean beetle and the grasshopper, and all yielded negative results. This confirms the reports of other workers (3, 5, 8) who were unable to transmit the virus with a number of the species recorded here. The number of plants on which insects fed ranged from 5 to 66, and the numbers of insects allowed to feed on infected plants and later transferred to healthy ones were from 5 to 13 per plant. Although further tests may show that some of them, as well as others not tested, may be vectors of the bean-mosaic virus, it appears that they play little part in the wide-spread dissemination of the dis-

[4] Insects mentioned under this heading identified by C. W. Kearns.

ease under field conditions. This is especially true of the two species of flea beetle and the clover leaf hopper, both of which are usually very numerous on beans in the early part of the season, and of the Mexican bean beetle and the grasshopper, which appear later. If any of these insects were vectors of the virus, all of the susceptible bean varieties would possibly be 100 per cent infected by the end of the season.

DISCUSSION

The evidence presented shows that although aphids are not so numerous on beans as many other species of insects, they are responsible for the dissemination of mosaic under field conditions. Because of the difficulty in collecting aphids by means of the usual field methods and in finding them except by careful examination of the individual plants, they have not been heretofore suspected as vectors in the transmission of bean mosaic under field conditions.

Because of the fact that aphids do not readily colonize on beans, many workers have concluded that they were not responsible for the field dissemination of the disease. Although Nelson (5, 6) is of the opinion that aphids are able to transmit bean mosaic, he believes that none of those tested by him are concerned with the wide-spread dissemination of the disease in Michigan. Likewise, Pierce and Hungerford (8) were not able to show any connection between the spread of mosaic and the prevalence of aphids under Idaho conditions, although they showed them to be vectors.

From the evidence presented on the prevalence of aphids in relation to the spread of mosaic (Table 3), it hardly seems possible that so few aphids could account for the wide-spread dissemination of the disease as the results indicate. Although the numbers of aphids recorded in the weekly examinations both in Colorado and Virginia do not indicate an abundance of these insects for any particular date, it is probable that their numbers may often have been larger between these periods. It is important to remember that there apparently was a continuous drift of winged aphids into the field and that those present on the plants when the counts were made had not been there over 72 hours or, as indicated in the rearing studies, they would have been dead. In view of this fact, it seems reasonable to conclude that the weekly aphid population counts represented the number of aphids present on a particular date rather than the total weekly aphid population. In other words, the total population for any week would be considerably larger than indicated by the weekly counts. It is likely that an average of 1 aphid per plant recorded for any single date may have represented the presence of 7 or more per plant for the entire week.

Most of the aphids found on beans were the winged migrant forms; and, since the bean is not a preferred host, the migration is likely to be continu-

ous. Furthermore, aphids in any appreciable numbers coming from other hosts and feeding on beans probably would not remain there but a short time, but would migrate to more favored hosts. Before doing so, however, they may become infective and feed on a number of plants in various parts of the field, transmitting the virus and thereby causing a rather general spread of the disease. Since Fajardo (3) has shown that a single aphid is able to transmit the virus, it is not unlikely that even a small population moving frequently from plant to plant could transmit the disease to many plants in the course of a short time. Furthermore, since the ability to transmit the bean mosaic virus is not necessarily confined to a single species of aphid, but the virus can be transmitted by many species, it appears that they are responsible for the field dissemination of the disease.

Both Elmer (2) and Fajardo (3) believe the mealy bug, *Pseudococcus maritimus*, is a vector of the virus. In northern climates these insects occur mainly in greenhouses, but they have been reported as occurring out of doors, especially on the roots of certain perennial crops. It is believed that they are not apt to be found feeding on beans in the field and would not, therefore, play a part in the field dissemination of the disease.

Smith and Barker's (9) studies indicate that the bean yellows, transmitted by *Empoasca* sp., is apparently unlike the common bean mosaic in this country. Previous to the report of Drake (1) *et al.*, who found over 50 different species of aphids able to transmit the yellow dwarf of onions, aphids in general were considered quite specific in the transmission of the various virus diseases. Since 11 different species of aphids have been shown to transmit bean mosaic, it is quite likely that others also will prove to be vectors. Furthermore, extended field studies on the insect fauna of beans may reveal many more species of aphids present on beans than those recorded in a single year's survey.

SUMMARY

The studies presented show that aphids are not found in large numbers feeding on beans in the field. The greatest infestation usually occurs about 10 days after the beans are above ground. As the season progresses, the populations decrease.

Fourteen species of aphids were found on beans in the field and it is likely that a careful search would reveal many others. Not all of the species found were tested, since, in some cases, they were too few. It is probable that the relative percentages of the different species found on beans depend to a large extent on the crop growing in close proximity to bean fields.

Positive transmission of the bean-mosaic virus was proved for 11 species of aphids collected from 17 different host plants. Only one of the aphid

species tested (*Neothomasia populicola*) collected from the cottonwood tree gave negative results.

The spread of the bean mosaic in the field is dependent on the prevalence of aphids, as well as on the amount of primary infection appearing early in the season.

Transmission studies with 10 species of insects other than aphids commonly found feeding on beans yielded negative results.

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FACTORS INFLUENCING INFECTION OF BARLEY BY LOOSE SMUT

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INTRODUCTION

In an earlier paper the writer (4) presented the results of experiments designed to determine the influence of certain environmental factors on the development of loose smut in barley. At that time it was taken for granted that there existed only one species of loose smut attacking barley. The wide differences in results obtained on the control of this loose smut by means of chemical seed treatments were attributed to differences in the manner of natural inoculation by the causal fungus on different varieties of barley. Tapke (6, 7) has since described another loose smut of barley and has named it *Ustilago nigra* Tapke.[2] Two of the chief characteristics distinguishing it from *U. nuda* are its ability to cause infection when spores are applied to the seed and its amenability to control by surface disinfectants.

The existence of two loose smuts of barley, one amenable to control only by the hot-water treatment and the other by ordinary seed disinfectants, explains many of the conflicting data presented in recent years on the loose smut of barley and its control. The work of developing varieties of barley resistant to this newly discovered loose smut demands a knowledge of what environmental conditions are most conducive to its development, so that supposedly resistant varieties may be tested under these conditions.

Since previous experiments dealing with the effects of environmental factors during the early life of the plants on the incidence of barley loose smut leave one in doubt as to which loose smut was involved, further work of this nature was done with *Ustilago nigra*.

REVIEW OF LITERATURE

Considerable work has been done by various investigators on the effect of environmental conditions on infection by other cereal smuts. This includes studies of the factors influencing the germination of both the smut spores and the cereal seeds. Attempts have been made to correlate slow seed germination and tardy host-seedling emergence with abundant smut infection. Much of this work has dealt with wheat and the organisms causing bunt. The smuts of oats and sorghum and the covered smut of barley also

[1] The writer gratefully acknowledges the advice and assistance of Dr. V. F. Tapke throughout the progress of these investigations.

[2] Some investigators are in doubt as to whether this smut should be accorded specific rank. In this paper, however, it will be referred to as *Ustilago nigra*.

have received considerable similar study. The salient literature on this subject has been reviewed by Reed and Faris (5). Loose smut of barley, on the other hand, has been largely omitted in studies of this kind. As previously stated, such studies as have been made on this smut are, in the light of Tapke's discovery, now of uncertain value because it is not definitely known which loose smut of barley figured in the experiments, or whether both may not have been present.

MATERIALS AND METHODS

Alpha barley was the principal variety used in these experiments, although Wisconsin Pedigree No. 5 was employed in a few cases. The first lot of Alpha used for work with *Ustilago nigra* unfortunately (unknown to the writer) carried some natural inoculum of both species of loose smut. This made the results obtained harder to interpret. The second lot of Alpha seed was raised in the greenhouse and was smut-free. The smut used for inoculation was obtained in the greenhouse, from mature plants that were allowed to cure and dry thoroughly before the smutty heads were removed. The latter were rubbed through a 60-mesh sieve and the powdered smut kept in a glass vial at 7° C.[3] until used.

Two methods of inoculating the seed were used. The first was a modification of the "spore suspension-vacuum method" employed in Germany and described by Haarring (2). A spore suspension was made by thoroughly shaking up 1 gram of spores in 1000 cc. of 1 per cent dextrose solution. This amount of spore suspension was poured over 250 grams of seed and the container again thoroughly shaken. Then by means of a small motor-driven vacuum pump the barley, together with the spore suspension, was subjected to 35 inches of vacuum for 20 minutes while being shaken occasionally to facilitate escape of air. The vacuum was then released, the liquid drained off, and the seed allowed to dry overnight. It was then stored for about 2 days at 25° C. and about 90 per cent relative atmospheric humidity.

The other method employed consisted simply in dusting the seed with dry spores and then incubating it as above.

Soil Moisture

Exact percentages of soil saturation are difficult to secure and maintain and no serious attempt was made to adjust the soil to predetermined exact percentages of its water-holding capacity. The aim rather was to obtain a relatively dry soil, a relatively wet soil, and one about half saturated. The water-holding capacity of the soil was first determined and then its actual moisture content. Sufficient water then was added to a given weight of soil to bring it up approximately to a given percentage of saturation. In

[3] The Centigrade temperature scale is used throughout this paper.

order to prevent puddling in the case of the very wet soil, the final increment of water was not added until after the seed had been planted. The soil cans were covered until after emergence to prevent evaporation.

In experiments carried out in the greenhouse bench, adjustments of the dry and medium-wet soils were secured by several tests. The wet-soil adjustment was obtained after planting by soaking the soil in the bench twice daily. The other sections were covered with heavy canvas until the seedlings emerged. Water was withheld from the dry and medium-wet sections until the plants were in the second-leaf stage. During this period the wet section was watered heavily daily.

Soil Temperature

A range of soil temperatures was secured by means of the soil-temperature tanks previously described (3). These tanks, although still the same in principle, had been improved by the addition of electric refrigeration and more efficient control instruments. In certain experiments controlled temperature chambers were employed. The low temperatures were automatically maintained by means of a compressor, refrigerating coils, and multiple expansion valves.

The air temperature in the greenhouse and consequently also the soil temperature in the benches ranged from 15 to 25° C., which, as will be shown later, is a range more or less favorable for infection by *Ustilago nigra*.

The plants grown in the soil cans suspended in the tanks were transferred to the greenhouse bench when they had reached the second-leaf stage.

The plants in the temperature chambers were grown in glass-covered flats. On emerging they were subjected to artificial illumination until large enough to transplant. Some time after the plants had been transferred to the bench, the regular daylight period was supplemented by electric lights, so that an 18-hour day resulted, thus hastening maturity.

Soil Composition

The possible influence of soil composition on infection was studied by growing artificially inoculated Alpha barley in 3 kinds of soil: (1) river sand, (2) Keyport silt loam from the Arlington Experiment Farm, and (3) a rich black "garden soil" to which leaf mold and powdered sheep manure had been added. The sand, loam, and "garden soil" had water-holding capacities of 21, 35, and 50 per cent, respectively, and the moisture content of each was adjusted to about 65 per cent of its respective water-holding capacity. The first experiment was carried out in deep flats and the second in 3 sections of greenhouse bench. The greenhouse temperature ranged from 10° to 20° C. during the first experiment and from 15° to 25° C. during the second.

SOIL REACTION

To obtain a range in soil reaction, dilute sulphuric acid or calcium carbonate was added to a good grade of top soil with an initial pH of 6.6. By preliminary tests it was determined how much acid or calcium carbonate to add to a given weight of this soil to produce the desired soil reaction. After the proper amount of calcium carbonate or acid had been added, the different lots of soil were shoveled over frequently during a period of several weeks and tested each time for reaction by means of the La Motte colorometric test and also by means of the W. A. Taylor slide comparator. The process of adjusting these lots of soil to different reactions was a long, laborious one requiring several months.

The first experiment was carried out in deep flats and the other two in sections of greenhouse bench.

EXPERIMENTAL RESULTS

Soil Moisture and Temperature

The results from experiments involving differences only in soil moisture are shown in table 1. In the first experiment, in which the maximum percentage of saturation was only 75 per cent, there were no significant differences in the percentages of infection obtained in the 3 lots of soil. In the second experiment significantly less smut developed in the very wet soil than in the other 2 lots. The same held true in the third experiment in the case of Alpha barley, but in Wisconsin Pedigree No. 5 the differences were barely significant. In none of these experiments were there any significant differences between the percentages of smut obtained in the soils containing low and medium amounts of moisture, although in earlier experiments (4) a minimum of soil moisture had seemed most conducive to smut development.

Additional data on the effect of differences in soil-moisture content on infection were obtained in connection with experiments involving also soil-temperature differences and are presented in table 2.

The lowest percentages of infection, especially at 5° and 30° C., and, with two exceptions, also at the other temperatures, occurred in the very wet soil. The plants grown to emergence in relatively dry soil at 5° C., contained much more smut than did the plants in the wetter soils at the same temperature. This was not the case at the higher temperatures, and is suggestive of the "interaction of factors" mentioned by Reed and Faris (5).

Differences between 10 and 25° C. in soil temperatures during emergence had no great influence on the percentage of infection. The highest average percentages of infection were secured at 15 and 20° C., with moderate declines at 10 and 25°, a greater drop at 5° and the most pronounced decrease at 30°. In 4 cases excessive soil moisture, combined with high or low temperature,

TABLE 1.—*Effect of soil moisture on infection by loose smut in Alpha and Wisconsin Pedigree No. 5 barleys grown from seed artificially inoculated with spores of Ustilago nigra and sown in soil adjusted to different percentages of its water-holding capacity*

Experiment	Variety	Range of soil temperature	Soil saturation		Plants		
			After sowing	After emergence	Grown	Smutted	
No.		° *C.*	*Per cent*	*Per cent*	*No.*	*No.*	*Per cent*
			30	25	392	77	19.6
1	Alpha	10 to 20	50	45	381	74	19.4
			75	73	442	98	22.2
			33	20	271	258	95.2
2	Alpha	15 to 25	57	44	286	257	89.9
			85	[a]	270	174	64.4
			25	19	237	207	87.3
	Alpha	15 to 25	45	39	244	207	84.8
			85	[a]	235	128	54.5
3							
	Wisconsin		25	19	284	203	71.5
	Pedigree	15 to 25	45	39	282	209	74.1
	No. 5		85	[a]	266	174	65.4

[a] Kept near saturation by watering heavily daily.

eliminated smut. Although in several cases rather high percentages of infection (59 and 73 per cent) occurred at 5°, due, possibly, to an unobserved irregularity in temperature, it is evident that 5° and 30° are much less conducive to infection than are the intermediate temperatures.

The data in series I (Table 2) are more or less affected by the presence of an unknown amount of smut due to *Ustilago nuda* with which the seed was, to some extent, naturally inoculated. In order to determine experimentally the approximate amount of infection by *U. nuda* so as to be able to interpret the results, and also to compare the effectiveness of the two methods of inoculation used, the following experiment was carried out. A quantity of the Alpha barley seed in question was divided into 4 lots: Lot 1 was neither inoculated nor treated; lot 2 was dusted with dry spores of *U. nigra;* lot 3 was inoculated with spores of *U. nigra* by means of a spore suspension in vacuum; lot 4 was inoculated the same as lot 3 and then treated with a 1: 320 formaldehyde solution for 1 hour. Seeds of all 4 lots were sown in flats in a somewhat sandy soil adjusted to 50 per cent of its water-holding capacity. The flats were placed in 3 chambers automatically maintained at 5°, 15° and 30° C., respectively.

TABLE 2.—*Infection by loose smut (Ustilago nigra) in Alpha and Wisconsin Pedigree No. 5 barleys grown from seed artificially inoculated*[a] *and sown in soil maintained at different temperatures and percentages of saturation until after the second leaf stage*

Series	Variety	Percentage soil saturation	Average number of plants grown[b]	Percentage plants infected at a soil temperature of:					
				5° C.	10° C.	15° C.	20° C.	25° C.	30° C.
I	Alpha	30	104	28	80	94	90	48	10
		47	107	13	91	93	86	85	3
		75	128	0	59	84	85	71	2
		Total or average	339	13	77	90	87	69	4
II	Alpha	35	106	73	85	92	97	79	19
		55	204	31	85	95	97	91	22
		90	16	7	32	22	58	56	0
		Total or average	326	41	78	88	92	84	19
III	Wisconsin Pedigree No. 5	35	73	59	60	81	79	52	4
		55	142	32	48	73	82	60	10
		90	22	0	50	71	65	47	0
		Total or average	237	40	52	75	80	53	7

[a] The seed in Series I contained some natural inoculation.

[b] At each combination of temperature and moisture.

The plants in the 30°, 15°, and 5° chambers emerged in 2, 6, and 28 days, respectively. They were kept in the different chambers under lights for several days after emergence, and then transferred to the greenhouse bench where they were grown to maturity. The data obtained are shown in table 3.

It is interesting to note that at 15° C. the higher percentage of infection resulted from inoculating the seed with dry spores and not, as might be expected, from applying a spore suspension in vacuum. This probably is due to the much heavier spore load put on the seed by the dry-spore method. The infection obtained after the seed had been treated indicates that it was naturally inoculated with *Ustilago nuda* to the extent of about 3 to 6 per cent. A comparison of these results with those from noninoculated, nontreated seed shows about the same amount of natural inoculation with *U. nigra*. The marked decrease in the amount of infection by *U. nigra* at 5° and 30°, compared with that at 15°, is outstanding.

In order to compare *Ustilago nigra* with *U. nuda* as to the effects of soil temperature before emergence upon the incidence of infection, seed of Alpha barley inoculated at flowering time with spores of *U. nuda* was included in the same experiment with seed of the same variety inoculated by dusting

TABLE 3.—*Loose smut in Alpha barley grown to emergence at 5°, 15°, or 30° C. from seed naturally inoculated to an unknown extent with both loose smuts and either not inoculated further or inoculated artificially with spores of U. nigra by two different methods with or without subsequent treatment in formaldehyde*

Inoculation and treatment	Data from plants grown to emergence at:								
	5° C.			15° C.			30° C.		
	Grown	Smutted		Grown	Smutted		Grown	Smutted	
	No.	*No.*	*Per cent*	*No.*	*No.*	*Per cent*	*No.*	*No.*	*Per cent*
[a]	150	9	6.0	150	21	13.9	140	14	10.0
[b]	647	31	4.8	621	559	90.0	614	113	18.4
[c]	758	68	9.0	670	585	87.3	590	107	18.1
[d]	125	5	4.0	120	7	5.8	111	3	2.7

[a] Not inoculated and not treated.
[b] Dusted with dry spores and not treated.
[c] Inoculated with a spore suspension under vacuum and not treated.
[d] Inoculated as in [c] and then treated in a 1: 320 formaldehyde solution for 1 hour.

with spores of *U. nigra*. The soil had a water-holding capacity of 37 per cent and was 50 per cent saturated. Six temperatures from 5 to 30° C. were used. Comparative results are shown in table 4. *U. nigra* caused a heavy infection —85.2 to 92.5—at temperatures from 10° to 25° C., inclusive, but produced only 12.6 and 2.6 per cent infection at 5° and 30°, respectively. *U. nuda*, however, produced the heaviest infection at 30° and almost as much at 5°. The data indicate that infection by *U. nuda* is not greatly affected by soil temperature during emergence, thus distinctly distinguishing it in this respect from *U. nigra*.

The possible effect of environmental factors after emergence on the incidence of smut has been suggested (4). To throw more light on this, seed of Alpha barley inoculated with spores of *Ustilago nigra* was sown in soil-temperature tanks maintained at 5°, 13°, and 30°, respectively. At emergence one third of each lot of plants was transferred to each of the other two temperatures, where the plants were grown until just before the heads appeared. The plants kept at 5° progressed so very slowly that it was found necessary finally to raise the temperature to 10° to bring about growth. This was done 3 months after planting. After the plants were almost in the boot they were kept at room temperature until smut data were taken, as shown in table 5.

It seems that the inhibiting effect of a soil temperature of 30° C. during emergence was not offset by a change after emergence to 13° C., a tem-

TABLE 4.—*Comparison of Ustilago nigra with U. nuda as to the influence of soil temperature before emergence on the incidence of infection in Alpha barley*

Soil temperature	Results from plants grown from seed inoculated with:					
	Ustilago nigra			*Ustilago nuda*[a]		
	Grown	Infected		Grown	Infected	
° *C.*	*No.*	*No.*	*Per cent*	*No.*	*No.*	*Per cent*
5	127	16	12.6	17	9	52.3
10	130	118	90.8	29	16	55.2
15	120	111	92.5	29	13	44.8
20	132	113	85.6	27	12	44.4
25	115	98	85.2	21	9	42.9
30	78	2	2.6	16	9	56.3

[a] Inoculated in the flowering stage.

TABLE 5.—*Loose-smut infection in Alpha barley as affected by soil temperatures before and after emergence of the seedlings grown from seed inoculated with spores of Ustilago nigra*

Transfer number	Soil temperature		Infection results			Items compared	Odds
	Before emergence	After emergence	Total plants	Infected plants			
	° *C.*	° *C.*	*No.*	*No.*	*Per cent*		
1	30	30	338	16	4.7	1 and 2	1: 200
2	30	13	301	2	.7	1 and 3	1: 50
3	30	5[a]	302	10	3.3	2 and 3	1: 430
4	13	30	307	91	29.6	4 and 5	Maximum
5	13	13	343	213	62.1	4 and 6	1: 640
6	13	5[a]	318	144	45.3	5 and 6	1: 2000
7	5	30	324	128	42.6	7 and 8	1: 25
8	5	13	13	173	55.3	7 and 9	1: 100
9	5	5[a]	308	96	31.3	8 and 9	Maximum

[a] Later raised to 10° C. to induce growth and heading.

perature supposedly more favorable for smut development. Instead, there was a significant decrease in the percentage of infection, seemingly due to this change. No explanation is offered for this erratic result.

The transfers from 13° to both 30° and 5° seemed to yield more logical results, as both caused highly significant decreases in the percentages of infected plants.

Both lots of plants transferred from a soil temperature of 5° at emergence showed significantly greater percentages of smut than the lot remaining at 5°.

The growth of the latter plants was retarded and their development was abnormal. This was true also of the plants transferred to 5° from the two other temperatures. On the whole the results from the experiment, especially those obtained from an initial temperature of 13°, seem indicative of the probability that environmental conditions after emergence influence the development of smut caused by *Ustilago nigra*. It should be borne in mind that the above changes applied only to the roots of the plants. It is possible that environmental changes involving the entire plants would produce more striking results. Equipment for such a study was not available.

In taking infection data on the above experiments it was observed that those plants grown to emergence at a soil temperature of 5° or 10° very frequently produced sound heads on the main tillers, while the secondary tillers bore infected heads. This seemed to indicate that infection occurred after the plants had been transplanted to another soil temperature. It is generally accepted as fact that invasion of the barley seedling by *Ustilago hordei* does not occur after the first green leaf has pushed through the coleoptile. To determine whether or not this is true also of *U. nigra* the following experiment was carried out.

Seed of Alpha barley containing some natural inoculation and in addition inoculated artificially with spores of *Ustilago nigra* was sown in flats that were kept in constant-temperature chambers at 5° and 30° until after the first leaf had emerged from the coleoptile. The soil had a water-holding capacity of 30 per cent and was 50 per cent saturated. The seed was sown on March 1. Seed of this same lot, not artificially inoculated, was included in a parallel series. Simultaneously, some soil mixed with viable spores of *U. nigra* was stored in each chamber along with a quantity of spores in a glass dish. When the first leaf was through the coleoptile, the plants grown from artificially inoculated seed were divided into two lots. In lot 1 the seedling roots and stems were washed clean in running water and then the plants were transplanted to the greenhouse bench. In lot 2 they were transplanted without washing. The seedlings grown from noninoculated seed also were divided into two lots. In lot 3, before transplanting, the bases of the seedlings were blackened with spores kept in the same chamber in which the seedlings had been grown. In lot 4 the seedlings grown at 5° and 30° had packed about their bases spore-infested soil that had been kept at 5° and 30°, respectively, during the period of emergence. The seedlings from the 30° chamber were transplanted on March 8, while those from the 5° chamber were transplanted March 22. Unfortunately, on the night of March 13, 3 days before the seedlings emerged, the compressor failed and the temperature in the 5° chamber rose from 5° at 3 p. m. to 26° at 9 a. m. The flats were transferred to a 10° chamber until 2 p. m., when the 5° temperature was restored. Therefore, for 23 hours the temperature was above 5°. This most

probably accounts for the high percentage of infection in the plants grown from inoculated seed in the 5° chamber as shown in table 6.

TABLE 6.—*Infection with loose smuts in Alpha barley grown from artificially inoculated and noninoculated seed at initial temperatures of 5° C. and 30° C. and transferred in the first-leaf stage to the greenhouse bench (15° C. to 25° C.), half of the seedlings from inoculated seed being first washed thoroughly and those from noninoculated seed being either blackened with spores or transferred to spore-infected soil*

Conditions at planting		Conditions at transplanting	Infection results		
Temperature	Artificial inoculation		Total plants	Plants smutted	
° *C.*			*No.*	*No.*	*Per cent*
5	spores	[a]	178	151	84.8
5	spores	[b]	185	113	61.2
5	none	[c]	157	7	4.5
5	none	[d]	158	7	4.4
30	spores	[a]	186	5	2.7
30	spores	[b]	266	7	2.6
30	none	[c]	135	0	0.0
30	none	[d]	136	2	1.5
15–25	spores	not transplanted	80	56	70.0
15–25	none	not transplanted	79	4	5.0

[a] Seedlings thoroughly washed.
[b] Seedlings not washed.
[c] Seedlings dipped in spores.
[d] Seedlings transplanted to spore-infested soil.

Instead of depressing the percentage of infection, washing the seedlings at transplanting increased it consistently and significantly, compared with unwashed seedlings. That no invasion of the seedlings took place after the first leaf had emerged, that is, after transplanting, is indicated by the low percentage of heads smutted when the seed was not inoculated. Most of these heads, in color and shape, were typical of infection by *Ustilago nuda*, especially those grown to emergence at 30°.

Effect of Soil Composition

The experiments involving different kinds of soil yielded mostly negative results (Table 7). In series I the percentage of infected plants grown in "garden soil" seemed significantly less than that of plants grown in sand or Keyport silt loam, but this significant difference was not duplicated in series II, although the average per cent of infection here also was slightly lower. An analysis of the detailed data from the experiment as a whole indicates that infection is not greatly influenced by soil composition.

TABLE 7.—*Loose smut infection in Alpha barley grown from seed artificially inoculated with spores of Ustilago nigra and sown in three kinds of soil—(a) river sand, (b) Keyport silt loam and (c) black "garden soil"*

Series	Soil	Plants			Heads		
		Grown	Infected		Grown	Infected	
		No.	*No.*	*Per cent*	*No.*	*No.*	*Per cent*
I	Sand	449	325	72.4	564	405	71.8
	Loam	473	315	66.6	570	379	66.5
	"Garden soil"	397	193	48.6	557	244	43.8
II	Sand	235	219	93.2	551	513	93.1
	Loam	169	157	92.9	576	539	93.6
	"Garden soil"	204	182	89.2	593	502	84.7

Soil Reaction

In the first experiment in which soil reaction was the variable, a significantly higher percentage of smutted plants developed in the acid soil than in the basic soil (Table 8). Differences between results obtained in neutral and acid soils and neutral and basic soils seemed of no significance statistically.

In the second experiment the results were the reverse of those obtained in the first one. A significantly lower percentage of smutted plants developed in the extremely acid soil than in either neutral or basic soil. Differences in results in the two latter soils were not significant. It is possible, however, that had many of the plants that failed to survive in the extremely acid soil been brought to maturity the results would have been different. It is probable that most of the plants that died were those previously weakened by smut invasion.

In the third experiment the acid soil of the second experiment had its pH raised from 3.8 to 4.7 by the addition of lime, and another planting was made later in this modified soil, as well as in soil of pH 7.7. As in the previous experiments, 8 replications were used. The differences in the results obtained on the 2 soils were not significant. The above 3 experiments as a whole failed to show any consistent effect of soil reaction on infection. It is evident that *Ustilago nigra* can develop in plants grown in soil that is beyond the acid tolerance of barley. In studies involving breeding for resistance or seed treatments soil reaction, therefore, is not likely to be an important factor.

DISCUSSION

In general, the reaction of *Ustilago nigra* to soil temperature in the infection of barley appears to be similar to that of *U. hordei* as determined by

TABLE 8.—*Loose smut infection in Alpha barley grown from seed artificially inoculated with spores of Ustilago nigra and sown in soil adjusted to different degrees of acidity or alkalinity*

Experiment number	Soil reaction	Plants			Heads		
		Grown	Infected		Grown	Infected	
	pH	*No.*	*No.*	*Per cent*	*No.*	*No.*	*Per cent*
I[a]	4.2	275	173	62.9	300	180	60.0
	7.0	363	189	52.1	424	205	48.3
	8.0	409	178	43.5	491	192	39.1
II[b]	3.8	127	51	40.2	532	183	34.4
	6.9	206	191	92.7	677	592	87.4
	8.1	215	189	87.9	677	574	84.8
III	4.7	243	211	86.8	453	397	87.6
	7.7	285	231	81.1	689	520	75.5

[a] Carried out in flats. If unheaded plants, omitted in calculations, were counted as noninfected the resulting percentages would be 42, 40, and 44 for acid, neutral and basic soils, respectively.

[b] In this experiment the plants had to be transferred from the acid soil to other soil to make continued growth possible. Many plants failed to develop.

Faris (1); that is, relatively low percentages of infection at 5° and 30° and fairly high percentages from 10° to 25°, with the optimum around 15°. The optimum temperature is somewhat higher than that for infection by bunt as determined by several investigators, and a trifle lower than that for the smuts of oats (5). As in the case of some of the other cereal smuts, the percentage of infection by *U. nigra*, under certain conditions, is depressed by excessive soil moisture during the period of emergence. This, undoubtedly, is due to oxygen deficiency. On the other hand, *U. nigra* seems to show no consistent response to differences in soil reaction in its infection percentages, while the covered smuts of barley and oats do (4, 5). It is probable that in experiments by various investigators reported prior to Tapke's work, the barley "loose smut," shown to be affected by environmental conditions, was *U. nigra*. Tapke (7) has demonstrated the reasonableness of this assumption.

SUMMARY

Soil with a high percentage of saturation proved generally unfavorable for infection by *Ustilago nigra*, especially at 5° and 30°, while relatively dry soil favored infection at 5°. At the other temperatures differences in soil moisture from 30 to 55 per cent saturation did not appear to influence infection.

The cardinal temperatures for infection by *Ustilago nigra* were found to be: Minimum below 5°, optimum 15° to 20°, and maximum above 30° C. Fairly high percentages of infection were secured at soil temperatures of 10°, 15°, 20°, and 25° C. *U. nuda,* on the other hand, showed little if any significant reaction to temperature.

Plants grown to emergence at 30° and then changed to a soil temperature of 13° C. showed less infection than those kept at a soil temperature of 30° C. until near heading. However, plants transferred at emergence from a soil temperature of 13° to 30°, or 5° showed a highly significant decrease in the percentage of infection compared with those kept at 13°. Likewise, those similarly transferred at emergence from 5° to 13° and 30° showed significant increases in the percentages of infection, compared with those kept longer at the lower soil temperature.

Dusting the seed with dry spores of *Ustilago nigra* resulted in heavier infection than inoculating the seed by means of a spore suspension in vacuum.

Results from experiments, in which emerging barley seedlings were inoculated with spores and with spore-infested soil, indicate that infection by *Ustilago nigra* does not occur after the first leaf has emerged.

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RELATION OF NITROGEN–CARBOHYDRATE NUTRITION OF STAYMAN APPLE TREES TO SUSCEPTIBILITY TO FIRE BLIGHT[1]

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This paper reports a study of the relation of succulence and hardness of apple shoots to infection by *Erwinia amylovora*. Differences in metabolic status with resultant differences in chemical and structural constitution, and in disposition to infection were induced by application and nonapplication of $Ca(NO_3)_2$.

Horticulturists and pathologists observed early that application of nitrogenous manures enhanced the susceptibility of pear and apple trees to spontaneous and experimental fire blight. Orchard experiments corroborated these observations (23, 28, p. 467, 29). Recently, in a study of environal factors disposing apples to fire blight, Shaw (25) made a few preliminary pot experiments with partial control of soil nutrients. Shoots grown in "sand-soil" blighted more severely than those grown in sand with sodium nitrate added. It was concluded that soil nutrients had a distinct effect on the susceptibility of Northwest Greening shoots and slight effect on the susceptibility of Wealthy shoots.

Experiments here reported involve more complete control of nutrition than any yet reported for potted trees inoculated with *Erwinia amylovora*. The reader interested in a bibliography is referred to recent publications on fire blight that give extensive citations of earlier papers (14, 22, 24, 25, 34).

MATERIALS AND EXPERIMENTAL PROCEDURE

The trees, root grafts of the Stayman variety, received from a commercial nursery in the fall of 1933 by G. T. Nightingale, were placed at the writer's disposal April 1, 1934 (18). On December 1, 1933, after several weeks in cold storage, the roots were thoroughly washed and pruned, leaving only old roots 0.5 in. or more in diameter. The tops also were pruned, leaving several buds, and an axis approximately 15 inches long.

The trees were then planted 4 per pot in the 2-gallon percolator urns used in the Chicago soil nutrient-temperature tanks (12). After covering the drain holes with glass wool, the pots were nearly filled with white nitrogen-free quartz sand, fine enough to pass a 1.0 mm. but not a 0.5 mm. sieve.

The trees were stored 2 months at 35–38° F., meantime receiving weekly applications of minus NO_3 ($-N$) solution (Table 1) sufficient to flush the

[1] This study was supported in part by a grant from the Rockefeller Foundation to the University of Chicago.

TABLE 1.—*Composition of nutrient solution*[a] *(partial volume molecular concentration of salts used)*

	$Ca(NO_3)_2$	KH_2PO_4	$MgSO_4$	$CaCl_2$
Complete, plus NO_3 (+N)	0.0090	0.0045	0.0045	
minus NO_3 (–N)		0.0045	0.0045	0.0090

[a] The pH value of these solutions was adjusted to 4.5 by addition of conc. H_2SO_4.

substrate. Distilled water was added as needed to keep the substrate moist.

On January 31, 1934, the trees were moved to a greenhouse, where the temperature was regulated to approximately 65–70° F. during the day and 60–65° F. at night. Daily applications of $-NO_3$ solution sufficient to flush the sand were made. Weekly, each pot was washed with several liters of distilled water, after which $-NO_3$ nutrient solution was applied immediately. All pots received two applications of $+NO_3$ nutrient solution between January 31 and February 15.

After bud development commenced, all but one bud per tree were removed from one lot of plants (8 pots, 32 trees) and all but 2 buds per tree were removed from the other lot (7 pots, 28 trees).

On April 10, 1934, all pots were transferred to tanks in another greenhouse. No attempt was made to control air and soil temperature, or humidity. During April, day temperature was 65–70° F.; night temperature, 60–65° F. During May, June, and July on dry, sunny days the temperature rose to 120° F. At no time, however, did the trees manifest water shortage.

On April 13, 1934, it was apparent that the shoots were about to develop terminal buds and cease elongating. Consequently, 750 cc. per pot of $+NO_3$ nutrient solution was applied to the 8-pot group of trees, hereafter designated as the +N lot. (Table 1). Each pot of the 7-pot lot received 750 cc. of the $-NO_3$ solution (Table 1), hereafter designated as the –N lot. Following this treatment both lots were watered daily with acidulated tap water. There was prompt response in the +N lot to the single nitrate application. The leaves became greener in 24 hours. By the 27th of April, 1934, striking color and form differences were noticeable in the shoots of the +N and –N lots.

From April 27 to May 2 all but one shoot of both the +N and –N lots were inoculated[2] with a 24-hour culture of *Erwinia amylovora*[3] in nutrient broth by injection with a hypodermic syringe. Each shoot was inoculated in the middle of the second apical internode and in the basal internode.

[2] The writers were assisted in the original inoculation by Messrs. W. S. Cook, W. S. Phillips, and Miss Frances Jewett.

[3] The culture was obtained from Dr. H. R. Rosen.

The shoots, especially tips, of the +N lot were succulent and readily inoculated; those of the −N lot were woody and inoculated with difficulty.

In the +N group, after blighting of the tip, one or more lateral shoots developed from the new wood. These were inoculated June 6. Following death of their tips was similar development of new lateral shoots from the first lateral shoot. These were inoculated July 13.

On June 11 half the trees of each lot were given an additional application of $+NO_3$ solution. In the −N lot this treatment, with shading, was an attempt to break dormancy and to initiate enlargement of the apical and basal cankers. In the +N lot this treatment was applied to determine whether increased diameter growth would lead to girdling of the axis by the basal cankers.

After August 1 the pots were kept moist with nitrate-free tap water with occasional application of $-NO_3$ solution, left in a cool greenhouse until January 1, 1935, and then transferred to cold storage.

Late in March, 1935, the trees were transferred to the greenhouse as in 1934. This time, however, the +N and the −N lots received biweekly applications of 750 cc. of the $+NO_3$ and $-NO_3$ solutions, respectively, and daily applications of acidulated tap water (pH 4.5), with occasional thorough flushing of the pots, to lower the concentration of the accumulating salts.

On April 22, after trees of both lots had developed succulent shoots, the apical internodes of some of them were inoculated.

Thereafter, during April, May, June, and July, each pot of the +N and −N lots received, daily, 500 cc. of $+NO_3$ and $-NO_3$ solution, respectively. By June 7, shoots of the −N lot had ceased elongation. Shoots of both lots were inoculated in the second apical internode. Those of the −N lot were placed in sunshine to further carbohydrate synthesis and hardening, while those of the +N lot were shaded part of the day. Contrasting uncontrolled weather factors in these experiments during spring and summer of the 2 years of 1934 and 1935, there were fewer sunny days, average and maximum temperatures were lower, and humidity was higher in 1935 than in 1934.

In 1934, both control and inoculated shoots were cut off, photographed, and histological examinations made after fixing them in Flemming's weaker fixative and embedding in paraffin. In 1935, shoots of each lot were wounded and fixed at definite intervals in order to determine rates of wound-periderm formation. The results of these experiments will be reported elsewhere.

RESULTS

At the time of first inoculation in 1934, shoots of the −N lot of plants had ceased elongating, developed terminal buds, were hard throughout their

entire length, and highly colored with anthocyanin. Leaves were hard and yellowish green. The average length was 6.7 inches and the average number of nodes was 9.9 (Table 2).

Microscopic examination of sections of the $-N$ axis revealed an abundance of starch in the pith, xylem, rays, pericycle, and inner cortex. The middle and lower internodes showed more starch, more thick-wall parenchymatous and sclerenchymatous elements, and more accompanying atrophy of protoplasts than the upper internodes.[4]

Following the 1934 inoculations, none of the $-N$ shoots developed leaf-killing or stem-girdling lesions in either the top or basal internodes. Apical lesions were small after 48 days, averaging less than $\frac{1}{4}$ inch. Basal lesions were even smaller.

The single application of $+NO_3$ to half the trees in the $-N$ lot led to some diameter increase and elongation of internodes, but did not break their dormancy. Tip and basal lesions increased slightly. In one instance the tip lesion became $\frac{3}{4}$ in. long, but without killing leaves or girdling the axis.

Following inoculation in 1935, 3 shoots in this lot developed apical lesions $\frac{1}{4}$ to $\frac{3}{4}$ in. long. Other than these lesions, all trees in the $-N$ group reacted the same in the 1935 as in the 1934 experiments.

At the time of the first inoculation in 1934, the $+N$ shoots were still elongating with no terminal buds. Upper internodes were very soft but lower ones had begun to harden and develop anthocyanin. The leaves, especially the upper ones, were large, tender, and dark green. Shoots averaged 16 inches in length and 14 internodes.

Contrasted with corresponding sections of the $-N$ axis, the $+N$ tissue showed less starch, less thick-wall parenchyma, fewer sclerenchymatous elements, and fewer atrophied protoplasts. The $+N$ lower internodes resembled $-N$ upper internodes.

Following apical inoculation, an average of 33 per cent of the average length of $+N$ shoots had blighted at the time the lesions became inactive. Maximum killing involved 45 per cent of the shoot. Final readings (Table 2) were made after 46 days. By this time many lesions had been inactive at least 16 days.

Lateral shoots that developed after death of the original shoot tip averaged 10 inches in length, with 9 internodes. Following apical inoculation

[4] The gradual disappearance of the protoplast in maturing phellem, fibers, stone cells, tracheids, and vessel elements may be considered atrophy of the protoplast, that is, of the cell proper, even though accompanied by wall hypertrophy. Unfortunately the term cell is applied indiscriminately to the protoplast, to the protoplast plus its wall, and to the wall plus the space once occupied by the protoplast. Atrophy of the protoplast is a permissible description.

TABLE 2.—*Inoculation experiments with shoots of Stayman apple trees of differing succulence and hardness following application and non-application of* $Ca(NO_3)_2$

Year 1934	Status of shoot at time of inoculation	Date of inoculation	No. of trees	No. of shoots	Length of shoot in inches at time of inoculation		Size of lesions after 46 days			
							Non-girdling non-leaf-killing in 1/16 in.		Girdling leaf-killing in per cent of length of shoots	
							Tip	Base	Tip	
					Max.	Average			Max.	Average
	–N; terminal bud formed	4/28–5/2	28	55	18	6.7	3 × 2	2 × 1.5	0	0
	+N; no terminal bud formed (1)	4/28–5/2	32	31	25	14		5 × 3	45	33
	Lateral from blighted shoot (1); no terminal bud formed (1a)	6/6	32	31	15	10			100	25
	Lateral from blighted lateral (1a); no terminal bud formed	7/13	32	31	10	3.3			30	18
1934	1935									
–N	–N; no terminal bud formed	4/22	3	3	4	3				100
+N	+N; no terminal bud formed	4/22	3	3	6	5				100
–N	–N; terminal bud formed	6/7	15	15	10	6.6	3 × 2		0	0
+N	+N; no terminal bud formed	6/7	16	16[a]	33	21			90[b]	59[b]

[a] 1 failure in a 12-inch shoot.

[b] Still progressing after 48 days.

23 per cent of their average length blighted, and, in a few cases, the entire shoot. However, such lesions did not penetrate the older wood.

Similarly, lateral shoots developed from the above blighted lateral shoots. These averaged 3.3 inches in length with 6.7 nodes. After apical inoculation 18 per cent of the average length of these shoots was blighted.

Basal lesions of the $-N$ shoots averaged $3/16 \times 5/16$ in. Two involved $\frac{1}{2}$ to $\frac{3}{4}$ the circumference of the stem, but none girdled the axis or killed leaves.

Following the additional application of $+NO_3$ solution to half the $+N$ lot, the basal lesions increased with the increased axis diameter and attained an average sixe of $5/16 \times 7/16$ in. Two induced local hypertrophy equal to 10 per cent of the diameter of the axis without, however, leading to other fire blight symptoms. Dormancy in the central shoot was not broken by this treatment.

The 1935 experiments corroborated those of 1934, showing even more striking contrast between the $-N$ and $+N$ shoots. This was partly due to the increased NO_3 application and partial shading of the $+N$ plants and partly to the lower average temperature, greater cloudiness, and higher humidity—factors all favoring succulent development.

Thus, in 1934, the noninoculated $-N$ control shoot ceased elongating by May 25, after attaining a length of 18 inches; the equivalent $-N$ shoot ceased by June 11, after attaining a length of 25 inches. In 1935 $-N$ control shoots stopped elongating June 6 at a length of 20 inches, while control $+N$ shoots were still elongating July 25, they having attained at that time a maximum length of 34 inches with 36 nodes.

In 1934, tip lesions in inoculated $+N$ shoots had ceased activity by June 11, that is, after 46 days, though most of them ceased after 30 days. In 1935, similar tip lesions were still advancing on July 25, 48 days after inoculation—an average of 59 per cent of the shoot length having been killed. Maximum killing was 27 inches, or 90 per cent of the shoot.

Inoculation on April 22, 1935, of a few very young actively growing shoots of both $-N$ and $+N$ lots, resulted in killing the entire shoot back to the old wood. The average length of the $+N$ shoots was 5 inches; that of the $-N$ shoots, 3 inches.

Histological study of inoculated shoots revealed very limited bacterial invasion in the tip and basal lesions of the $-N$ lot and in basal lesions of the $+N$ lot. In all cases the lesion was well walled off with wound periderm. The parasite was found in all tissues of the axis. Its location in the tissues and gross injuries to the middle lamella, primary and secondary walls, and protoplasts of succulent tissues is essentially that noted by other investigators (7, 14, 21, 24, 25).

DISCUSSION

Succulence and Disposition[5]

The experimental results indicate that disposition of Stayman apple shoots to fire blight is correlated with maturity of the inoculated part (14, 23, 24, 25, 29). If, at the time of inoculation, inoculated and adjacent tissues have ceased cell division and enlargement and have matured into thick-wall constituents, the relatively woody[6] shoot is negatively disposed to infection by *Erwinia amylovora* and, therefore, is considered resistant. If, on the other hand, there is cell division and enlargement and little maturation of tissues, the relatively soft succulent shoot is positively disposed to infection and is considered susceptible. Behavior of all lesions on non-elongating shoots and basal lesions in +N shoots corroborate the former statement, apical lesions in elongating shoots the latter. These results harmonize with earlier reports on other varieties (23, 29). The most susceptible varieties apparently produce and maintain the greatest proportion of succulent thin-wall tissues abounding in living protoplasts.

In the 1934 experiments, tip lesions in the +N lot did not progress far down the stem; the maximum amount of shoot blighted was 45 per cent; the average, 35 per cent. Most lesions on the main axis were inactive after 30 days; those on the lateral axis, after 15 days.

During the 1935 experiments additional nitrate applications, partial shading, lower temperatures, and higher humidity favored greater succulence. Under these conditions a maximum of 90 per cent and average of 59 per cent of the shoot was killed. After 48 days the lesion was still progressing into the apparently hard axis.

These observations indicate that, under favorable conditions, as the blighting advances down the axis, it progressively reactivates meristematic tissue in the matured hard parts of the axis. The cambium again gives rise to succulent thin-wall cells, and protoplasts of the heavy-wall cortical, pericyclic, phloem, xylem, pith, and ray parenchymas become active. Enzymatic activity thins the walls, and dividing cells give rise to new thin walls within the old ones. Regions, once poor in active protoplasts and thin walls, become relatively rich in both. Thus, progressive invasion by the parasite comes with increased succulence in the formerly hard, lower internodes of the axis. Similarly activated parenchymatous cells of the leaf and branch

[5] By disposition of the apple shoot and of *Erwinia amylovora* is meant that aspect of their constitutions rendering them either prepared or unprepared for infection.

[6] The terms woody and succulent are not used here solely as chemical concepts to designate the presence of lignin and of water respectively. Lüdtke (13) has pointed out that lignified or woody designates a total habit or status of particular tissues, organs, or plants, describing their morphological, physiological, ecological, and teleological properties. The same is true of the term succulent.

gaps are involved and, through them, petioles and branches are invaded. The susceptibility of the tree seems to depend upon the initial amount of succulent tissue formed during the main growth period, retention of succulent tissue as the axis matures, and resumption of meristematic activity by relatively inactive protoplasts.

These data indicate that the regulation of relative carbohydrate and nitrogen nutrition may be used experimentally to induce expression of succulence and susceptibility or woodiness and insusceptibility (11). Given identical environmental conditions, the metabolic status of trees supplied with the nutrient solution containing nitrate can be kept at a level favoring meristematic activity, cell enlargement, succulence, and susceptibility. By withholding or washing out nitrates, that metabolic level can be so changed that these come to an abrupt end (3, 4, 5). Shading or nonshading, etc., produce similar effects. The resulting + N or succulent plants are relatively low in carbohydrate and high in nitrate-free nitrogen compounds; the converse is true of the – N or woody plants (8, 19, 20, 26).[7]

In + N plants the balance of carbohydrate and nitrogen metabolisms, enzyme and hormone actions favors organic nitrogen and protoplasm synthesis (8, 19, 20, 26) with resultant cell division and enlargement. Protoplastic atrophy is delayed and infrequent; cell walls are thin; relatively few plasma-insoluble polysaccharides and nitrogenous compounds accumulate. The protoplasts are poor in starch, but rich in plasma-soluble organic nitrogen compounds. According to Lüdtke (13), young or thickening cell walls, in addition to pectic substances, cellulose, hemicellulose and soluble lignin and lignin-like compounds, contain plasma-soluble polysaccharides, amyloids, and intercelluloses that become converted into cellulose. Similarly, probable conversions accomplished in young walls are: interxylans to xylans, plasma-soluble intermediary stages of galactans and arabans to plasma-insoluble forms; and the final synthesis of pectins from linkages of polygalacturonic acids, galactose, and arabinose. If these interpretations are correct, primary and secondary walls and middle lamellae in course of formation contain enzymes that accomplish such synthesis and condensation, yielding end products characteristic of those structures.

In – N shoots carbohydrate and nitrogen metabolisms, enzyme and hormone action result in diminished synthesis of organic nitrogen and protoplasm (8, 19, 20, 26) and consequent checking of cell division, thickening of cell walls, and protoplastic atrophy. Plasma-insoluble polymerous carbohydrates and plasma-insoluble nitrogen compounds accumulate. Protoplasts are rich in starch, poor in plastic organic nitrogen compounds (16, 17). In the course of considerable secondary change constituents of the

[7] These publications, especially 8 and 26, contain extensive bibliographies on carbohydrate-nitrogen nutrition of apple trees.

middle lamellae and primary walls become less hydrated and less soluble. They contain, therefore, comparatively few pectic substances and a high proportion of the polymerous polysaccharides known as hemicelluloses (6, 15, 16), some cellulose (26), and the less soluble lignin (13, 26), and lignin-like compounds (13).

Other metabolic differences in + N and – N shoots exist, since the former are probably richer in auxins and cell-division-stimulation substances than the latter (35). The pH values also may differ because of altered proportions of tissues predominantly acid or alkaline (17). Environmental factors, such as air temperature (14, 19, 25), humidity (14, 20, 25), soil temperature (18, 25), and soil moisture (25), may be limiting in altering metabolic status as expressed through altered meristematic activity of genotypically identical plants. Thus, these factors influence relative rate of wound periderm formation (20, 25) and also disposition of apples to infection by *Erwinia amylovora* (25).

The query arises: what host characteristics favor infection? Hard, woody tissues of genotypically susceptible varieties and succulent tissues of genotypically insusceptible varieties may contain substances that inhibit the parasite. Examinations of punctures in such shoots show that introduced organisms do not increase appreciably before the wound is healed. Fewer bacteria and less gum are formed during the period required for wound periderm formation than during an equivalent period in a favorable site. Usually a band of apparently unaffected cells lies between the advancing edge of the bacterial mass and the periderm (25), indicating that the bacteria are first stopped, and then periderm formation follows. Its formation appears to be favored by conditions unfavorable to the parasite, and is more a regenerative (25) than it is the primary defensive reaction it has been interpreted to be (14).

The presence of abundant water in the intracellular spaces of succulent tissues favors invasion in providing a physical and nutritive medium for bacterial extension. However, if water were the limiting factor, suffused tissues of any plant should be susceptible to invasion by *Erwinia amylovora*. It seems probable that certain physical and chemical characteristics, such as the presence of soluble carbohydrates and nitrogen compounds and host hormones concomitant with wateriness of tissues favor propagation of bacterial thalli, as well as host cells.

Causal Complex of Metabolic Status and Disposition

The metabolic status of a shoot prior to and at time of inoculation is important in disposing it to infection. This status is conditioned by a causal complex of internal and external factors (9, 10, 11). The internal factors include the hereditary constitution and past experience of the shoot, and

correlative influence of other parts of the plant. Heredity determines the range of metabolic possibilities. Stayman is neither as suceptible or insusceptible as other apple varieties (24). At the time of inoculation certain internodes and tissues were conditioned not only by existing temperature, humidity, and other environmental factors, but by previously induced effects of these. The single application of nitrates to certain trees in 1934 produced effects evident not only in 1934, but in 1935. The past-experience factor is the result of all internal and external past factors that affected a given part prior to a given moment.

Correlative influences active at the time of inoculation are evident in the effect of root tips on the inoculated shoot. The root tips absorb the available nitrates, reduce them to nitrites and ammonium compounds, oxidize carbohydrates and their derivatives, synthesize amino acids, polypeptids, and transportable proteins, and thus affect the metabolism and disposition of the shoot (3, 4, 5, 19, 20, 26, 31).

After inoculation, blighting of a shoot generally led to development of a susceptible shoot from a lateral bud when the remainder of the plant was phenotypically insusceptible. Blasting of the terminal bud led to removal of its inhibitory influence through hormones upon the development of lateral shoots (2, 27, 30, 35). At the moment of inoculation the influence of this correlative factor has become part of the past experience of the inoculated shoot.

In these experiments, prior to its use as inoculum, the disposition of the parasite was considered a constant. Thereafter environmental changes must have significantly affected the organism when inoculated into tissues of such differing constitution as those in – N and + N shoots.

Before conclusions can be drawn relative to susceptibility to *Erwinia amylovora*, the particular plant must be grown under conditions favoring maximum vegetative growth and succulence. These factors for the apple are: application of nitrogen, limited light exposure, high humidity, moderate air and soil temperature, high soil moisture, low concentration of mineral nutrients, and a soil pH of approximately 4.5 (18, 20, 32, 33). In these experiments the potential susceptibility of the Stayman variety may not have come to full expression, since some factors influencing the trees were not conducive to metabolism that leads to maximum meristematic activity and accompanying succulence.

The Course of Injury in Infected Tissues

There is no agreement among recent competent investigators as to the nature of primary injury, mechanisms inducing it, and the later course of pathic events in the tissues (7, 14, 21, 24, 25).

Punctured succulent tissues suffer a rupture of walls and protoplasts, and from shock of adjacent unpenetrated cells; integrative properties of the

healthy protoplast are lost; and enzyme systems through unregulated catalysis often damage host tissues further and produce changes favoring invasion. In certain rosaceous plants, especially the stone fruits, there is a tendency toward progressive self-injury with copious gum formation following even simple mechanical wounds. Disruption of lignin and lignin-like substances in which the polymerous carbohydrates of walls are embedded (13) expose them to digestive and oxidative changes. Altered permeability as well as broken cell membranes make the wound abound with protoplastic contents. Thus a favorable medium is provided for the parasite in potentially susceptible hosts. Unless trauma leads to release of substances inhibiting the infective agent, a wound in succulent tissue provides better ingress than one in woody dehydrated tissue. In the course of these experiments it was found that in succulent tissues the wound was still filled with fluid 15 minutes after inoculation, while that in woody tissues was dry.

In non-wounded tissue the parasite must find or create self-favoring conditions. It is known that *Erwinia amylovora* is able to enter through the unbroken surfaces of nectaries or natural openings and establish itself in initially intact tissues. Whether the parasite *per se* possesses capacities to bring about directly all the destruction of middle lamellae, walls, and protoplasts noted in infected tissues, is not known. It may inflict a primary minor physical injury as a result of which cell processes become unregulated, initiate autolysis, and bring about the course of pathic events characteristic of tissue invaded by *Erwinia amylovora*. The gummy mass in which the bacterial thalli are embedded might be derived entirely from disturbed cells, and inhibit their prompt, healthy functioning. This together with imbibitional and osmotic forces (14) of the thalli might further injure the protoplast, alter its permeability, and account for the plasmolysis noted as early evidence of injury in infected tissues. Complete enclosure of cells (21, 7) in this mass and resultant interference with free influx and outflow of metabolic materials and by-products (24) leads to their injury and death. Dissolution of middle lamellae and plasmodesmata preliminary to such engulfment may be due entirely to enzymatic action of the disturbed wall and protoplast, though from observations of infected tissue (7, 14, 21, 24, 25) it seems more likely that the parasite physically or preferably chemically initiates such disorganization. Miller (14) reports that nutrient medium in which *Erwinia amylovora* was grown contained in low concentrations hydrolyzing enzymes that destroy the middle lamella of carrot. This capacity alone or in conjunction with heightened activity of enzyme systems in the injured protoplast and wall would further augment existing damage.

Whether nonregulated enzyme systems of the host, or enzymatic and mechanical destructive forces of the parasite, or both, play the critical rôle in initial host injury, the metabolic and structural status of host tissue

is important in conditioning phenotypic expression of genotypically determined capacity to develop susceptibility or resistance.

CONCLUSIONS

1. Shoots of Stayman apple trees grown in quartz sand were rendered succulent and woody, respectively, through the application of either plus or minus nitrate nutrient solutions, and inoculated with *Erwinia amylovora*.

2. Actively growing succulent shoots were susceptible. Shoots in which tisses had matured to the extent that they could not be restored to meristematic activity were not susceptible.

3. Application of nitrates with particular light, temperature, and moisture conditions favored a metabolic status resulting in succulence and susceptibility to infection of Stayman apple shoots by *Erwinia amylovora*. Nonapplication of nitrates under the same conditions favored early cessation of meristematic activity, resulting in woodiness and resistance to infection.

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THE DISTRIBUTION, CAUSE, AND RELATIVE IMPORTANCE OF CRANBERRY FRUIT ROTS IN MASSACHUSETTS IN 1932 AND 1933, AND THEIR CONTROL BY SPRAYING

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The control of cranberry fruit rots in Massachusetts and New Jersey is a problem of primary commercial importance. Shear (4), in 1920, estimated that the annual loss from fruit rot averaged at least 25 per cent of the total cranberry crop of the United States. This estimate was reaffirmed by Shear, Stevens, and Bain (5) in 1931. Studies on various phases of the problem of the control of fruit rots have been carried on for some 30 years. Material progress has been made, but much yet remains to be done. A recapitulation of the results up to 1931 is given in the publication just cited.

As a part of spraying and storage experiments carried on in 1932 and 1933, studies have been made on the distribution and relative importance on certain bogs, of fungi causing fruit rots of cranberries in Massachusetts, and on the degree of control of specific fungi by spraying. The bogs on which spray plots were located are widely separated and represent considerable diversity in local conditions. The bogs are designated as No. 1, 2, 3 in this article, S.E. and N.E. refer to plot location. The varieties on these bogs are mainly Early Black and Howes, the sorts most extensively grown in Massachusetts. Two minor varieties, Middleboro and Holliston, also are included.

In these experiments duplicate plots were laid out that received 1, 2, and 3 applications of spray material, respectively, during the season, with adequate check plots. The results are given for specific individual plots. All plots had an area of 1/20 acre, except those on bog 1, which were 1/50 acre. A Bordeaux mixture made up with granular $CuSO_4$ and chemically hydrated lime, using a 4–6–50 formula with one pound of potassium fish-oil soap for each 50 gallons of water, was applied to most of the plots. Some mercurial fungicides were used on a few plots in 1932. A small experimental power sprayer was employed, capable of producing a maximum pressure of 100 pounds per square inch at the pump, when spraying. The pressure at the nozzle was probably not more than 75–80 pounds. The spray was applied at the rate of 300–400 gallons per acre at each application. The first application was made when the first flowers were just beginning to open. When only one application per season was given it was at this time. A second application was made as soon as the petals had fallen from most of the flowers. A third application was made about the 10th to the 15th of August or about three weeks after the second. The mercurial sprays were prepared

by dissolving one pound of the material in 100 gallons of water. The time, rate, and method of application were the same as for Bordeaux.

The percentage of total spoilage due to a particular fungus on a given plot was determined by a method essentially the same as that described by Stevens and Bain (6, p. 650). In calculating the results the modification suggested by them (7, p. 809) was used. In this work a quarter-barrel stock sample was made up as the berries were harvested by taking one to three quarts of berries from each picking box belonging to a particular plot. These stock samples were stored in the basement of the Cranberry Experiment Station at East Wareham, Massachusetts, for use in subsequent tests.

The first determination of spoilage of berries from all plots was made usually within 1 to 2 days and except in a single case, as stated below, never later than a week after the berries had been picked. Four-quart subsamples from each stock box generally were used and the percentage of spoilage was determined by weight. In September, 1932, the determination was made by count from 3-pint samples and in September, 1933, by weight from 1-quart samples. The percentage of rot present at harvest was not determined immediately for berries picked after October 15, 1932, the berries being allowed to stand until mid-November before sampling.

The causes of subsequent spoilage in the various lots were determined by the following procedure. About the middle of each month, from October to January, inclusive, a 4-quart subsample of sound berries was taken from each quarter-barrel stock sample and stored for 2 weeks in the basement, under the same conditions as the samples from which they were taken. Then the subsamples were carefully sorted and the percentage of spoiled berries determined. During the winter of 1933–34 the percentage of rot present in the quarter-barrel stock samples was determined at the middle of each month, when the subsamples for storage tests were sorted out. Cultures were made from spoiled berries from representative lots, when the subsamples were sorted out at the time of picking and also at the end of each 2-week storage test. The cultures were held at room temperature until the fungi fruited or developed sufficiently for identification. In this way the progress of spoilage in berries due to any specific fungus could be followed from the time the berries were picked until the end of the storage season on February 1. In 1932–33 determinations of the amount of rot present in stock samples at the middle of each month were not made, but the berries from the plots on one bog were screened the middle of October and from the other bog about the middle of November and the percentage of spoiled berries removed during screening was substituted for these values. For these two bogs, therefore, the progress of spoilage during the season can be followed and compared with that in 1933. This comparison is shown graphically in figure 1.

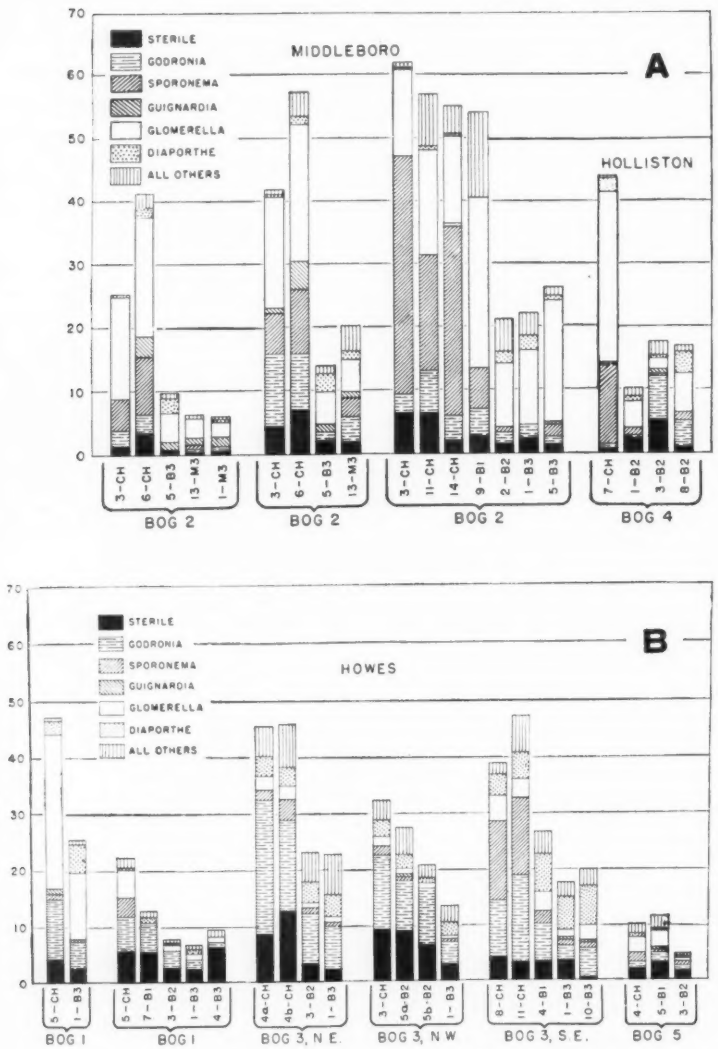

FIG. 1. A. A comparison of the total spoilage of Middleboro and Holliston cranberries from sprayed and check plots, calculated as percentage of yield of the respective plots. The proportion of total spoilage caused by different fungi and by sterile breakdown is shown by sections with special markings, as indicated in the legend. The left group of columns, of the three groups shown for Middleboro bog 2, represents spoilage in the 1932 crop to December 1, 1932; the middle group, spoilage in the 1932 crop to February 1, 1933; and the right group, spoilage in the 1933 crop to November 27, 1933; for Holliston, bog 4, the spoilage is for the 1933 crop to January 1, 1934. Plot numbers and treatment are shown below the columns, Ch—Check, B—Bordeaux, M—Mercurial. Figures following B indicate number of applications during season. B. A comparison of the total spoilage of Howes from sprayed and check plots on three bogs, calculated as percentage of total yield of the respective plots. The two columns of bog 1 at the left represent spoilage in the 1932 crop to February 1, 1933; all others are for the 1933 crop to February 1, 1934.

DISTRIBUTION AND RELATIVE IMPORTANCE OF VARIOUS FUNGI

The total amount of spoilage in berries from nonsprayed plots on any of the bogs studied is no greater than that reported in previous studies (5), except in Early Black from bog 3, where the total spoilage in 1933 amounted to 65 to 95 per cent of the crop. The various bogs differed widely, not only in the total amount of spoilage in berries from them, but also in the relative importance of the several fungi causing it. Early rots caused the greater part of the spoilage of berries from unsprayed plots of a majority of the bogs investigated during the seasons of 1932 and 1933. The most outstanding example is that of the Early Black on bog 3, in which they caused a loss of 60 to 85 per cent of the crop in 1933. There was also an unusually high proportion of loss caused by early rots in berries from nonsprayed plots on bog 2 in 1932 and 1933, and on bog 4 in 1933. Early rots were much less

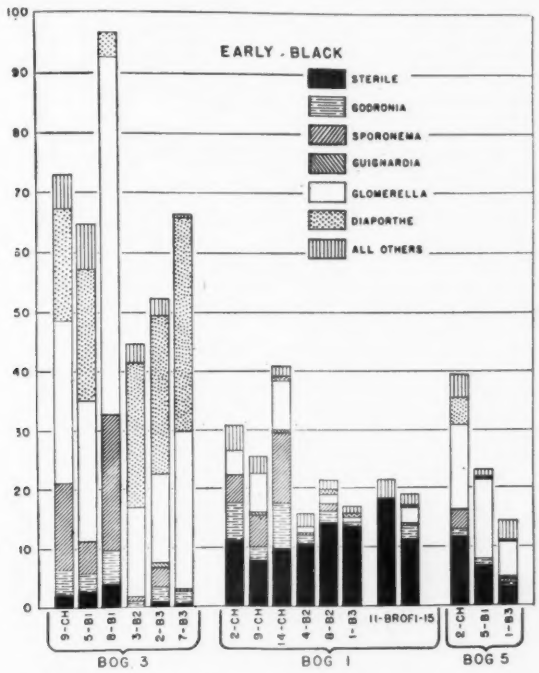

FIG. 2. A comparison of the total spoilage in Early Black cranberries from sprayed and check plots on three bogs, calculated as percentage of yield of the respective plots. The total height of each column represents the percentage of spoilage in the 1933 crop of bog 3 to November 1, 1933; and for the 1933 crop of bogs 1 and 5 to February 1, 1934. Total spoilage and proportion of loss caused by different fungi and by sterile breakdown, indicated as in figure 1. Plot numbers and treatment are shown below the columns, Ch—Check, B—Bordeaux.

important as causes of spoilage in the Howes from N.E. and N.W. plots on bog 3, in 1933, than either Godronia[1] or sterile breakdown.

Glomerella, Sporonema, and Diaporthe were the most important causes of early rots during 1932 and 1933. Glomerella was the principal cause of spoilage in berries from nonsprayed plots on bog 2 (Fig. 1, A), and of Howes on bog 1, in 1932 (Fig. 1, B); from plot 9 on bog 2, on bog 4 (Fig. 1, A), and in Early Black on bogs 3 and 5, in 1933 (Fig. 2). Although much less important on other bogs during these 2 years, it was never negligible, but was of minor importance in Howes from bog 3, in 1933.

On some bogs Sporonema caused as much or more loss than Glomerella. Rot caused by Sporonema resulted in a loss of 30 to 38 per cent of the berries of 3 nonsprayed plots on bog 2, in 1933. This is greater than that caused by all other causes combined. It was much less important on this bog in 1932, but, in 1933, was the most important single cause of loss in Howes from nonsprayed S.E. plots on bog 3. Although listed by Shear, Stevens, and Bain (5) under "Important Rot Fungi" and stated (p. 8) to be "A rather common cause of storage rot of cranberries in all cranberry sections" it was not of sufficient importance in their work during a 4-year test to be mentioned as a cause of spoilage (5, Fig. 35; 6, 7, 8); and, as shown on page 35, appeared in less than 5 per cent of the cultures up to November 1 during the 4-year period. Rudolph and Franklin (1) found Sporonema to be the least important of 4 fungi as a cause of spoilage in cranberries in 1916 and 1917. They found that most of the spoilage caused by this fungus was in the Early Black. Their results are expressed as percentage of spoiled berries and, although indicating the relative abundance of different fungi, have no absolute value in terms of percentage of spoilage of the storage sample, or in terms of crop loss.

In berries from most of the bogs studied during 1932 and 1933 Diaporthe caused less rot than either Glomerella or Sporonema. The loss caused by it was not more than 2 to 4 per cent, except in the Early Black from several plots on bog 3, in 1933, where it caused a loss of 18 to 35 per cent of the crop (Fig. 2). On these plots, except one, the loss caused by it equalled or exceeded that due to either Glomerella or Sporonema.

Guignardia was much less important as a cause of fruit rot, on the bogs studied during the 2 years, than any of the 3 fungi named above. It caused a spoilage of more than 1 per cent of the yield in berries from a plot on bog 2 only, in 1932. This fungus has been recognized as of appreciable importance as a cause of early rot in berries from Massachusetts. In the 1927 crop of Howes and McFarlin (5, Fig. 35) it caused the spoilage of approximately 11 per cent of the storage samples.

[1] Glomerella, Sporonema, Godronia, etc., refer to fungus species as published by Shear, Stevens and Bain (5).

It is evident that the importance of any given fungus varies independently of that of any other, and varies not only on different bogs but also on different parts of a particular bog. These variations in the local abundance of specific fungi must be due to local differences in conditions that favor the development of one fungus rather than another. High temperatures during the growing season promote the further development of whatever fungus becomes dominant on any particular bog or plot. The effect of various factors on the development of fungi in competition or association with one another, under field conditions, is at present not known. Among the factors concerned it seems probable that bog management, particularly with respect to the use of water, is very important. For example, bog 3 was reflowed in June, 1933, to which operation the large amount of spoilage in the Early Black on this bog was probably mainly due. Bog 1 was reflowed from June 12 to 14, 1932, which caused a high percentage of spoilage in the berries, as shown in the graph for plot 5 of Howes (Fig. 1, B). The winter flood on this bog was held until May 23, 1933. The total spoilage of berries from plot 5 (Fig. 1, B) in 1933 was less than half that of the previous year, despite much higher temperatures during May and June and the greater number of rainy days during July and August. The reduction in the amount of loss due to Glomerella in berries from plot 5, in 1933, as compared with the loss due to this fungus in 1932, is very striking.

CONTROL OF FRUIT ROTS BY SPRAYING

Spraying was beneficial on all bogs on which experiments were conducted in 1932 and 1933, although much variation in the degree of control on different bogs and even on different plots on a given bog was observed. In most instances the amount of rot in berries, at picking time, from plots that had been sprayed 2 or 3 times was reduced to 25 per cent or less of that found in adjacent nonsprayed plots. The benefit of spraying was evident, not only at this time, but continued throughout the storage season. In many instances 2 applications of 4–6–50 Bordeaux mixture[2] gave apparently as good control of rots as did 3. This, as well as other variations in the apparent degree of control of fungous rots, may be due in part to differences in the abundance of fungi on different plots. One application of Bordeaux usually caused some reduction in the amount of rot but was never so effective as 2, and in some instances apparently produced no effect. Two mercurial sprays, phenyl mercury acetate and ethyl mercury arsenate, used on bog 2 in 1932, were fully as effective as Bordeaux in controlling rots. In one instance only, that of Early Black on bog 3, the control or rots by spraying with Bordeaux was notably poor.

[2] Equivalent to 4–4–50 of the old formula when stone lime was used.

An analysis of the effects of spraying with reference to the control of specific fungi shows that, in some instances, spraying brought about little or no reduction in the amount of spoilage caused by certain fungi, while in other instances rots were controlled very effectively by the same treatment. Glomerella and Diaporthe were the fungi that were controlled least. The failure to control these organisms accounts for the notably poor results of spraying the Early Black on bog 3 mentioned just above. Also, spraying had no evident effect on the amount of spoilage due to Glomerella in the Middleboro variety from bog 2, in 1933. A similar relation with reference to Diaporthe was observed in Howes from S.E. plots on bog 3, in 1933. It is possible that in some instances that first application of spray may have been made somewhat later than it should have been to secure effective control of these fungi. Thus in 1933, it was estimated that 5 to 10 per cent of the buds of Early Black on bog 3 had opened before the first spray was applied. Also, on bog 2, in 1933, many of the flowers were in bloom when the first spray was applied. Studies made since then indicate that infection of flower buds by fungi causing fruit rots occurs at an early stage of bud development. Moreover, bog 3 was reflowed for 32 hours on June 9 and 10 and the first spray was not applied until June 22 so that a great deal of infection had probably taken place before the spray was applied. In all other instances spoilage due to Glomerella as well as to other fungi was greatly reduced by spraying, particularly by three applications. Glomerella was effectively controlled on bog 2, in 1932.

In both 1932 and 1933, spoilage due to Godronia was found very much less in berries from plots sprayed 3 times than from adjacent nonsprayed plots. This is particularly noticeable in a comparison of graphs for plots 3 and 6 with plot 5 on bog 2, in 1932; for plots 4a and 4b with plot 1 N.E., for plot 3 with plot 1 N.W., and for plots 8 and 11 with plots 1 and 10 S.E. of Howes on bog 3, in 1933; for plots 2 and 14 with plot 1 of Early Black, and for plot 5 with plot 4 of Howes on bog 1, in 1933. Two applications of spray, however, had little or no effect on the control of Godronia. This indicates that infection by this fungus occurs during the latter part of the summer and that a late spray is perhaps necessary for its control.

Although it was demonstrated by early experiments that spraying usually improves the keeping quality of cranberries in Massachusetts (2, 3), growers have not adopted the practice of spraying. The principal reason for this probably is that the generally good keeping quality of cranberries from most bogs makes it doubtful if there is sufficient benefit to warrant the cost of spraying. This is undoubtedly true for the majority of bogs in Massachusetts, but there are many bogs on which spraying might be done with profit. The amount of improvement obtained in 1932 and 1933 would make a material increase in the net income of the grower by reducing the

amount of rot in the field, in storage before shipment, and particularly in the reduction of adjustments and rejections as a result of spoilage after shipment. On bogs producing cranberries that are below the average in keeping quality, spraying should be adopted as a recognized practice in bog management.

SUMMARY

Early rots caused the greater part of the spoilage of berries from unsprayed plots of a majority of the bogs investigated during the seasons of 1932 and 1933.

Glomerella, Sporonema, and Diaporthe were the most important causes of early rots in 1932 and 1933. Sporonema was much more important as a cause of fruit rot than has previously been reported. Diaporthe caused a loss of 18 to 35 per cent of the crop from several plots on one bog in 1933.

The importance of any given fungus varies independently of that of any other and varies not only on different bogs, but also on different parts of a particular bog.

A single application of 4–6–50 Bordeaux mixture[3] was often ineffective and was never so effective as two applications.

Two and three applications of spray were beneficial on all bogs except one on which experiments were conducted in 1932 and 1933, although much variation in the degree of control was observed.

In many instances two applications of Bordeaux gave apparently as good control as three.

Two mercurial sprays, phenyl mercury acetate and ethyl mercury arsenate, used on one bog in 1932, were fully as effective as Bordeaux in controlling rots.

With reference to the control of specific fungi by spraying it was found that Glomerella and Diaporthe were controlled least, and Sporonema was effectively controlled by two or three applications of spray.

The failure to control rots in Early Black on one bog in 1933, by three applications of Bordeaux, is probably due to the fact that the bog was reflooded early in June, prior to the first application of spray.

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[3] Equivalent to 4–4–50 with stone lime.

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SPECIFICITY OF ACQUIRED IMMUNITY FROM TOBACCO–RING–SPOT DISEASES[1]

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INTRODUCTION

In the last few years a number of workers, including Thung (23), Salaman (19), Oortwijn Botjes (12), Kunkel (10), Ainsworth (1), Caldwell (2), Price (15), and McKinney (11), have presented evidence to show that plant tissues invaded by one strain of a plant virus are immune from infection by other strains of the same virus. The evidence also shows that this immunity is specific in that it does not extend to any of the viruses that are not related to the one used for immunization.

There are 4 virus diseases from which certain species of plants are known to recover and acquire immunity. Three of these, ring spot, green ring spot, and yellow ring spot, have been described previously (26, 24, 14). The fourth, which is designated as ring spot No. 2, was recently isolated by the writer. Since these 4 diseases are alike in many respects, it was suspected that their causal agents might be closely related. It was, therefore, decided to use the immune reaction as an aid in determining the relationship of these viruses and at the same time test the specificity of acquired immunity from the tobacco ring spots by including a number of other viruses which, either from previous work or because of differences in symptoms, host range, and properties, were considered to be entirely distinct from those of the ring-spot group.

SOURCE OF VIRUSES

The viruses used, together with the sources from which they were obtained, and references to descriptions in the literature, are listed in the tabulation on page 666.

The ring-spot virus obtained from Dr. Wingard is designated as ring spot No. 1 in order to avoid confusion with the virus isolated by the writer and designated as ring spot No. 2. Tomato-aucuba-mosaic virus is now recognized as a strain of ordinary tobacco-mosaic virus (10). Southern celery-mosaic virus has recently been shown to be a strain of cucumber-mosaic virus (16). Potato-vein-banding virus was reported by Chester (3) to be serologically identical with cucumber-mosaic virus. Potato-ring-spot virus is believed to be a strain of latent-mosaic virus.

[1] Published at the expense of The Rockefeller Institute for Medical Research, Princeton, N. J., out of the order determined by the date of receipt of the manuscript. This practice in nowise delays the publication of manuscripts printed at the expense of The American Phytopathological Society or other agency.

Virus	*Source*	*Literature citation*
Tobacco ring spot No. 1	S. A. Wingard	(26), (14), (17)
Tobacco green ring spot	W. D. Valleau	(24)
Tobacco yellow ring spot	W. D. Valleau	(24)
Tobacco ring spot No. 2	Recently isolated by the writer	
Tobacco mosaic	James Johnson	
Tomato aucuba mosaic	L. O. Kunkel	(10)
Cucumber mosaic	R. H. Porter	(13)
Southern celery mosaic	F. L. Wellman	(5), (25)
Potato vein banding	E. S. Schultz	(8)
Potato ring spot	E. S. Schultz	(9)
Tomato spotted wilt	From a grower in Oregon	(20), (21), (4)
Tobacco etch	W. D. Valleau	(8)
Tobacco severe etch	W. D. Valleau	(8)

DIFFERENTIATION OF THE RING-SPOT VIRUSES

The symptoms caused by most of the viruses used in the present study are so well known that a description of them need not be given here. A description will, however, be given of the symptoms of tobacco ring spot No. 2, since there is no previous reference to this disease in the literature. In addition, it is believed desirable to point out some of the similarities and differences in symptomatology between tobacco ring spot No. 1, green ring spot, and yellow ring spot.

Differentiation of Ring Spot No. 1, Green Ring Spot, and Yellow Ring Spot. The 3 diseases may be distinguished by the symptoms they produce in 2 host plants, tobacco (*Nicotiana tabacum* L. var. Turkish) and cowpea (*Vigna sinensis* Endl.). Yellow ring spot is distinguished from both ring spot No. 1 and green ring spot by the fact that it produces bright yellow, as well as necrotic, systemic lesions in tobacco, whereas the other 2 diseases produce only necrotic or pale yellow lesions. It also is characterized by the fact that old leaves of plants that have recovered from the disease are yellow in color, particularly on their margins, while leaves of plants that have recovered from either ring spot No. 1 or green ring spot are dark green. Ring spot No. 1 differs from green ring spot in producing more severe symptoms in tobacco, considerably more necrosis, and somewhat greater stunting. Plants recover from ring spot No. 1 earlier than from either green or yellow ring spot, suggesting that the virus of ring spot No. 1 moves more rapidly in tobacco than either of the other 2 viruses.

The primary lesions produced by ring spot No. 1 in cowpeas are usually solid necrotic spots and only infrequently fail to become completely necrotic, while those caused by green or yellow ring spot are, as a rule, necrotic only around their peripheries. The virus of ring spot No. 1 moves more rapidly

and causes more injury in the cowpea than the virus of either green or yellow ring spot. In a set of cowpea plants inoculated with one or another of the 3 viruses, all the plants infected with ring spot No. 1 were dead on the 9th day after inoculation, while those infected with green and yellow ring spot were alive on the 13th day.

Tobacco Ring Spot No. 2. In the summer of 1934, and on several subsequent occasions, there appeared in a greenhouse at Princeton a ring-spot disease in 1 or 2 plants in an otherwise healthy set of Turkish tobacco. The virus of this disease is readily transmitted by the leaf-rubbing method of inoculation. In tobacco it produces primary lesions consisting of zonate necrotic spots similar to but distinct from those produced by tobacco ring spot No. 1 (Fig. 2). The disease likewise produces systemic lesions of the same type, and plants affected systemically by it eventually recover in much the same manner that tobacco plants recover from tobacco ring spot No. 1 (14). On the other hand, the disease may frequently become localized, in which case the virus fails to reach the tip portions of the plant. It is usually possible to induce systemic symptoms and subsequent recovery by cutting back the plants or by fertilizing them heavily. Tobacco plants that have recovered from ring spot No. 2 are immune from a second attack of the disease.

Ring spot No. 2 was transmitted to *Nicotiana glutinosa* L., *N. langsdorfii* Weinm., *N. sylvestris* Spegaz. and Comes, tomato (*Lycopersicon esculentum* Mill.), bean (*Phaseolus vulgaris* L.), and cowpea, and was characterized by the production of primary and systemic necrotic lesions in these hosts. The subsequent behavior of the disease was not extensively studied.

Evidence was obtained that virus of ring spot No. 2 may be transmitted through seed of Turkish tobacco to a small percentage of progeny from diseased plants. A careful observation of approximately 5,000 seedlings grown from seed of plants that had recovered from the disease revealed no symptoms in any of them. The seedlings were then divided into lot of about 125 each, juice being expressed from each lot and tested separately for presence of virus by inoculation of 6 Turkish tobacco plants. Infection was obtained from only one of the 40 lots of plants, and in this instance symptoms typical for the virus of ring spot No. 2 were obtained. While the evidence for seed transmission is not conclusive, it is at least suggestive that the virus may occasionally be transmitted through seed of Turkish tobacco.

Tobacco ring spot No. 2 is similar in some respects to the necrotic virus disease described by Smith and Bald (22). A sample of the virus was sent to Dr. K. M. Smith who reported that he did not believe it to be identical with his necrotic virus nor with any of the other viruses in his collection.

TESTS FOR ACQUIRED IMMUNITY IN TURKISH TOBACCO AND NICOTIANA SYLVESTRIS

All the experiments to be reported on specificity of acquired immunity from tobacco-ring-spot diseases were repeated on one or more occasions. The results obtained from the repetitions were similar to, and served to confirm, the results obtained in the first experiments.

Ring-spot Diseases. Experiments were conducted for the purpose of determining whether or not plants that had recovered from any one of the 4 ring-spot diseases would be immune from either of the other 3. In one set of experiments, Turkish tobacco was used as a host plant and in another set *Nicotiana sylvestris.* Since similar results were obtained with these 2 hosts, they will be considered together.

For the experiments with Turkish tobacco, tip cuttings were taken from healthy control plants and from plants that had recovered from ring spot No. 1, from green ring spot, from yellow ring spot, and from ring spot No. 2, respectively. They were rooted in moist peat moss and sand and then potted in 4-inch pots. When they had attained a suitable size, 5 plants of each of the 5 groups were heavily inoculated with one of the ring-spot viruses by means of the rubbing method.

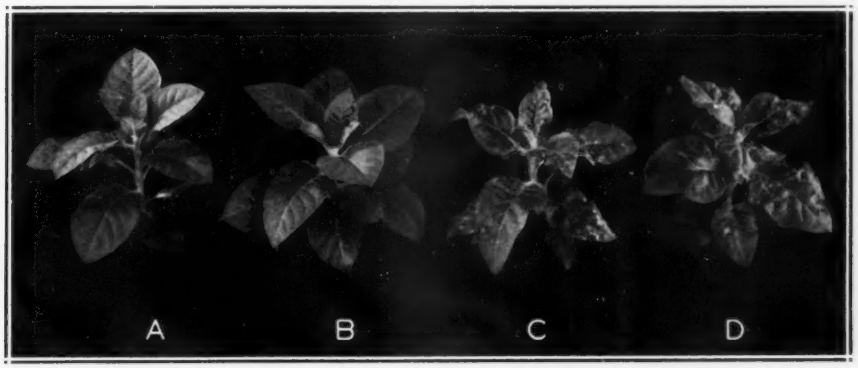
For the experiments with *Nicotiana sylvestris,* cuttings grown from recovered and from healthy plants were divided into sets each of which consisted of 5 plants that had recovered from ring spot No. 1, 5 that had recovered from yellow ring spot, and 5 controls. One set of plants was inoculated with ring spot No. 1, another with yellow ring spot, a third with green ring spot, and a fourth with ring spot No. 2.

TABLE 1.—*Summary of tests for immunity in plants of Turkish tobacco and Nicotiana sylvestris*

Virus used for inoculation	Tests with Turkish tobacco					Tests with *N. sylvestris*		
	Plants recovered from				Controls	Plants recovered from		Controls
	Ring spot No. 1	Green ring spot	Yellow ring spot	Ring spot No. 2		Ring spot No. 1	Yellow ring spot	
Ring spot No. 1	++[a]	++	++	0	0	++	++	0
Green ring spot	++	++	++	0	0	++	++	0
Yellow ring spot	+	+	++	0	0	+	++	0
Ring spot No. 2	0	0	0	++	0	0	0	0

[a] 0 = no protection, + = partial protection, ++ = complete protection.

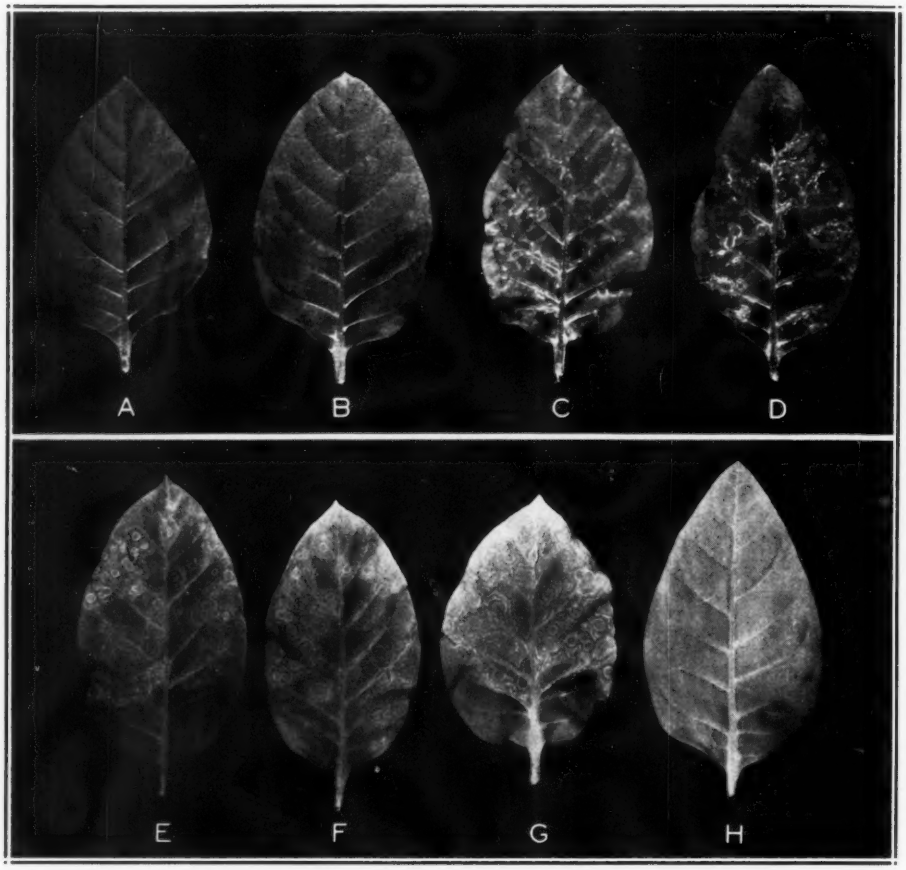
Table 1 summarizes the results obtained. Three of the viruses, ring spot No. 1, green ring spot, and yellow ring spot, gave protection against one another, but did not protect against ring spot No. 2. Plants that had recovered from the latter were likewise susceptible to the other ring-spot diseases, but were solidly immune from ring spot No. 2. The findings are illustrated in figures 1 and 2. Figure 1 shows the results obtained when ring spot No. 1 was used as inoculum, figure 2 (A to D) the results when green ring spot was used, and figure 2 (E to H) the results when ring spot No. 2 was used.



Photographed by J. A. Carlile

FIG. 1. Turkish tobacco plants grown from cuttings and inoculated with virus of ring spot No. 1. A. A plant that had recovered from ring spot No. 1. B. A plant that had recovered from green ring spot. C. A plant that had recovered from ring spot No. 2. D. A previously healthy plant. The photograph was taken 10 days after inoculation.

It will be noticed from table 1 that ring spot No. 1 gave complete protection against green ring spot and that green ring spot gave complete protection against ring spot No. 1, but that neither ring spot No. 1 nor green ring spot completely protected plants from infection with yellow-ring-spot virus. When plants that had recovered from ring spot No. 1 were inoculated with yellow-ring-spot virus, they showed no symptoms during the first 9 days after inoculation, but on the 10th day they exhibited a number of small, yellow, primary lesions. The lesions increased somewhat in size, and in a few instances developed partially necrotic edges. Systemic lesions were observed 40 days after inoculation and consisted of small yellow spots with zonate rings. As the plants became older, more and more yellow lesions appeared in the young leaves, so that eventually they were almost entirely covered by small yellow areas. At a still later stage the doubly infected plants recovered from the yellow-ring-spot disease and produced leaves similar in appearance to those produced by plants that had recovered from



Photographed by J. A. Carlile

FIG. 2. Turkish tobacco leaves inoculated with virus of green ring spot (A to D) and with virus of ring spot No. 2 (E to H). A, E. From a plant recovered from ring spot No. 1. B, F. From a plant recovered from green ring spot. C, G. From a previously healthy plant. D, H. From a plant recovered from ring spot No. 2. The picture was taken 10 days after inoculation.

yellow ring spot alone. Figure 3 illustrates the lesions obtained in *Nicotiana sylvestris* one month after inoculation.

When plants that had recovered from green ring spot were inoculated with yellow-ring-spot virus, they showed no symptoms for a month after inoculation. A few discrete yellow lesions then appeared in some of the inoculated leaves. Similar lesions developed later in the young noninoculated leaves. In other instances, no symptoms were produced within the period of more than 2 months that the plants were kept under observation.

Ring Spot and Other Diseases. Experiments were undertaken to deter-

Photographed by J. A. Carlile

FIG. 3. *Nicotiana sylvestris* plants grown from cuttings. Left, a plant recovered from ring spot No. 1 and inoculated with virus of yellow ring spot. Center, a previously healthy plant inoculated with virus of yellow ring spot. Right, a healthy control. Note the small lesions in the leaves of the plant on the left and compare with the large lesions present in the center plant. The picture was taken one month after inoculation.

mine, firstly, whether or not the ring-spot viruses would protect tobacco plants from infection with 9 other viruses, and, secondly, whether or not these other viruses would afford protection against the viruses belonging to the ring-spot group. The experiments were similar to those reported in the previous section.

The viruses used for inoculation of plants that had recovered from one or another of the ring-spot diseases were tobacco mosaic, aucuba mosaic, cucumber mosaic, celery mosaic, potato vein banding, potato ring spot, tomato spotted wilt, etch, and severe etch. In the reciprocal tests, the virus of spotted wilt was not included because of the difficulty of obtaining a thorough systemic infection with it in tobacco. Also, celery-mosaic and aucuba-mosaic viruses were not included because of their relationship to cucumber-mosaic and tobacco-mosaic viruses, respectively.

The results of the experiments may be summarized briefly as follows: The ring-spot viruses gave no protection against any of the other viruses tested, nor did any of these other viruses protect plants from infection with any of the ring-spot viruses. The symptoms of tobacco mosaic in plants that had recovered from ring spot No. 1, from green ring spot, or from yellow ring spot were milder than those produced in the control plants. A similar modification of tobacco-mosaic symptoms has been reported previously (14). The symptoms of etch in plants that had recovered from ring spot No. 1 were also milder than those in the control plants. In most instances, the lesions produced by the ring-spot viruses in plants infected with the other viruses tested were somewhat less numerous than those produced in the controls. Systemic symptoms of the ring-spot viruses were slightly delayed in plants

infected with severe etch as compared with the controls. In other respects, the symptoms produced by the ring-spot viruses in plants infected with the other viruses tested, and those produced by these other viruses in plants that had recovered from one or another of the ring-spot diseases, were no different from the symptoms produced in the control plants.

The conclusions to be derived from the results of these experiments may be summarized briefly as follows: Firstly, the fact that ring spot No. 1 and green ring spot protect completely against each other indicates that they are closely related strains belonging in the same virus group. Secondly, the fact that yellow ring spot protects completely against ring spot No. 1 and against green ring spot, together with the fact that both ring spot No. 1 and green ring spot afford a partial protection from yellow ring spot, suggests that the viruses of the 3 diseases are related. This conclusion is in accord with that reported by Chester (3), who found a close serological relationship between the virus of ring spot No. 1 and yellow ring spot. Thirdly, the virus of tobacco ring spot No. 2 must be considered as entirely distinct from all the other viruses tested. Finally, the failure of the ring-spot viruses to induce immunity against any one of 9 viruses outside the ring-spot group brings evidence for the specificity of the immune reaction.

Tests with Plants Recovered from both Ring Spot No. 1 and Ring Spot No. 2. An experiment was performed for the purpose of determining whether or not plants that had recovered from ring spot No. 1 and also from ring spot No. 2 would become immune from both diseases. Cuttings were grown from 5 plants that had recovered from ring spot No. 1, from 5 plants that had recovered from ring spot No. 2, and from 10 healthy plants. The 5 plants that had recovered from ring spot No. 1 and 5 of the control plants were inoculated with virus of ring spot No. 2. Similarly, the 5 plants that had recovered from ring spot No. 2 and the remaining 5 control plants were inoculated with virus of ring spot No. 1. The plants in all 4 sets became infected, developed symptoms, and in due time recovered. Five of the plants that had recovered from both ring spot No. 1 and ring spot No. 2 and the 5 plants that had recovered only from ring spot No. 1 were inoculated with virus of ring spot No. 2. Symptoms developed in the plants that had recovered from ring spot No. 1 alone, but not in the plants that had recovered from both diseases. The remaining 5 plants that had recovered from both ring spot No. 1 and ring spot No. 2 and the 5 plants that had recovered from ring spot No. 2 alone were inoculated with virus of ring spot No. 1. No symptoms developed in the plants that had recovered from both diseases, but the plants that had recovered only from ring spot No. 2 came down with a severe infection of ring spot No. 1. The results show that, while tobacco plants recovered from only one of the 2 ring-spot diseases are susceptible to the other, plants that have recovered from both diseases are immune from both.

DISCUSSION

The results reported here show that acquired immunity from tobacco ring spot is highly specific. Plants that have recovered from ring spot No. 1 are immune from this disease and from green ring spot, and they are highly resistant to yellow ring spot. They are not, on the other hand, immune from tobacco ring spot No. 2, tobacco mosaic, aucuba mosaic, cucumber mosaic, celery mosaic, potato vein banding, potato ring spot, tomato spotted wilt, etch, or severe etch. The results have likewise shown that the immunity acquired by plants that have recovered from ring spot No. 2 extends only to this disease and not to any of the others studied.

The failure of ring spot No. 1 to protect completely against yellow ring spot is of interest because it indicates a possibility that yellow ring-spot virus has associated with it a property peculiar to itself in addition to properties also associated with ring spot No. 1. The virus of the latter disease has the power to induce immunity from ring spot No. 1, but not a complete immunity from yellow ring spot, while yellow-ring-spot virus induces immunity not only from yellow ring spot but also from ring spot No. 1.

If the degree of protection afforded by the immune reaction may be taken as an indication of the closeness of relationship of plant viruses, then the evidence suggests that yellow ring spot is more closely related to green ring spot than it is to ring spot No. 1. Plants that have recovered from green ring spot appear to be less susceptible to yellow ring spot than are plants that have recovered from ring spot No. 1. To a certain extent this conclusion is supported by other lines of evidence. While the necrotic lesions produced in cowpea by green- and yellow-ring-spot viruses are practically identical, they differ from those induced in the same host by virus of ring spot No. 1. It has been shown (24) that green- and yellow-ring-spot viruses are transmitted through seed of tobacco to as much as 15 per cent of progeny from recovered plants. On the other hand, no evidence has been obtained that the virus of ring spot No. 1 is transmitted through seed of Turkish tobacco (18, 14, 7), although it has been shown to be transmitted through seed of petunia (6).

It is of considerable interest that there are at least 2 distinct virus diseases from which tobacco plants regularly recover, and that in the case of both of these the plants are immune from a second attack. It may be of some significance that these diseases are much alike in their symptomatology, in their host ranges, and in other ways. However, if there is a connection between recovery from a disease and some particular characteristic of the disease, it remains to be discovered. On the other hand, it is apparent from a study of tobacco ring spot No. 1 and tobacco ring spot No. 2 that either of these diseases interferes little or not at all with the other. A plant that has recovered from one is highly susceptible to the other and, once infected,

develops severe symptoms, which are followed sooner or later by recovery. Plants that have recovered from both diseases are immune from both.

SUMMARY

A virus disease of tobacco, designated as ring spot No. 2, was shown by means of the immune reaction to be entirely distinct from ordinary tobacco ring spot, designated as ring spot No. 1, and from green and yellow ring spot. Tobacco plants infected with ring spot No. 2 recover and develop a solid immunity from the disease. They do not become immune from tobacco ring spot No. 1 or from any of the 11 other virus diseases tested.

Ring spot No. 1 and green ring spot give complete protection against each other, but neither protects completely against yellow ring spot. On the other hand, yellow ring spot protects against ring spot No. 1 and green ring spot. These facts indicate that ring spot No. 1, green-ring-spot, and yellow-ring-spot viruses are closely related.

Ring spot No. 1, green ring spot, and yellow ring spot in tobacco gave no protection against tobacco-mosaic, aucuba-mosaic, cucumber-mosaic, celery-mosaic, potato-vein-banding, potato-ring-spot, spotted-wilt, etch, or severe-etch viruses. The results bring evidence for the specificity of acquired immunity from tobacco-ring-spot diseases.

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PYTHIUM GRAMINICOLUM AND P. ARRHENOMANES

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In a recent paper Carpenter (3), after a consideration of literature on species of Pythium responsible for root injury, more particularly to graminaceous hosts, set forth the conclusion that the parasite associated in Hawaii with growth failure of sugar cane (*Saccharum officinarum* L.)—a parasite he had first referred to as *P. butleri* Subr., and later as *P. aphanidermatum* (Eds.) Fitz.—was identical with both *P. graminicolum* Subr. and *P. arrhenomanes* Drechsl. Subramaniam's statement (12) that the fungus isolated in India from diseased roots of wheat (*Triticum aestivum* L.) agreed with the description and drawings of the Pythium destructive to sugar cane in Hawaii (2), together with Matthews' corroborative citation of the "*P. aphanidermatum* of Carpenter" as a synonym of *P. graminicolum* (9), was held to establish identity with the one species; while the inclusion recently by Rands and Dopp (11), of an actual culture isolated in Hawaii from diseased sugar-cane roots among the strains of *P. arrhenomanes* studied by them, was interpreted quite justifiably as establishing identity with the other. Not illogically, this dual identification was held to imply identity of the two species themselves; wherefore, it presumably remained only to determine which of the two had been described the earlier to decide upon the name by which the fungus might be correctly designated. As priority appeared to favor Subramaniam's description over my own, *P. graminicolum* was adopted as the valid binomial; *P. arrhenomanes*, at the same time, being reduced to synonymy.

If the dispositions thus made were to be found in satisfactory agreement with the facts of morphology, the resulting simplification in taxonomy would be more welcome even than the abasement of a species name somewhat indecent in its spiritual connotation. It can not be doubted that the identification of the Hawaiian fungus used by Rands and Dopp as *Pythium arrhenomanes* was altogether correct. The detailed study by these authors, of numerous strains of the species both in comparison with each other and with many strains referable to a dozen other species of Pythium, could hardly have failed to supply more than adequate information and experience. On the other hand, Subramaniam's judgment as to the scope of his species relative to the fungus set forth as a parasite on sugar cane in Hawaii, was apparently based solely on information conveyed in Carpenter's early account. With respect to what would seem to constitute the most decisive morphological feature concerned here, the origin and mycelial connections of the antheridia, this account unfortunately is somewhat less clear than

might be desired. In the illustrations accompanying it, the parts probably meant to represent antheridia show no indication of being set off by basal septa; and many of the elements thus meagerly differentiated are shown juxtaposed to the oogonium in a manner leaving doubt as to whether structural continuity with, or disappearance beneath, the female organ was intended to be depicted. A degree of ambiguity is thereby introduced that would seem to make reference of the description to any particular one of several likely species about equally uncertain. Comparison of Carpenter's figures with those illustrating the original account of *P. graminicolum* reveals so little correspondence in origin of antheridial branches that Subramaniam's ready acceptance of specific identity appears somewhat surprising. Nor is a closer agreement evident when comparison with regard to antheridial connections is extended to Matthews' figures of presumably the same fungus.

Since the culture on which the description of *Pythium graminicolum* was based has long been lost, it it fortunate that the description in itself leaves little uncertainty as to the proper application of the species. The close mycelial connection between oogonium and antheridia, evident in Subramaniam's figure, is paralleled in many cultures that R. D. Rands, beginning in 1927, isolated from diseased roots of sugar cane, together with numerous strains of various other forms, among which, as was intimated in my earlier account (4), *P. arrhenomanes* was abundantly represented. These cultures were, from the beginning, recognized as belonging to a species so distinct from *P. arrhenomanes* that confusion between the two was not expected. The species in question had become known to me some years earlier through a single culture received from B. A. Bourne, who had isolated it in Puerto Rico from a diseased root of sugar cane in connection with investigations reported by him (1) in 1924.

The relationship of parts in the sexual apparatus to the Puerto Rican fungus is illustrated in figure 1. This figure, it may be explained, was drawn in 1925; so that its preparation was influenced neither by Subramaniam's paper, which appeared 3 years later; nor by Matthews' book, issued 6 years later; nor, least of all, by considerations growing out of the issue raised in Carpenter's recent contribution. A parallelism in antheridial connections, with the sexual apparatus of *Pythium graminicolum* as figured by both Subramaniam and Matthews, is, I believe, so readily apparent as to require no further exposition. In appropriately irrigated material the fungus gives rise to lobulate zoosporangia and zoospores likewise corresponding well to the homologous structures of the wheat parasite. It was therefore assigned, together with the morphologically similar cultures obtained from sugar-cane roots in the southern United States, to *P. graminicolum* as soon as the description of that species made its appearance.

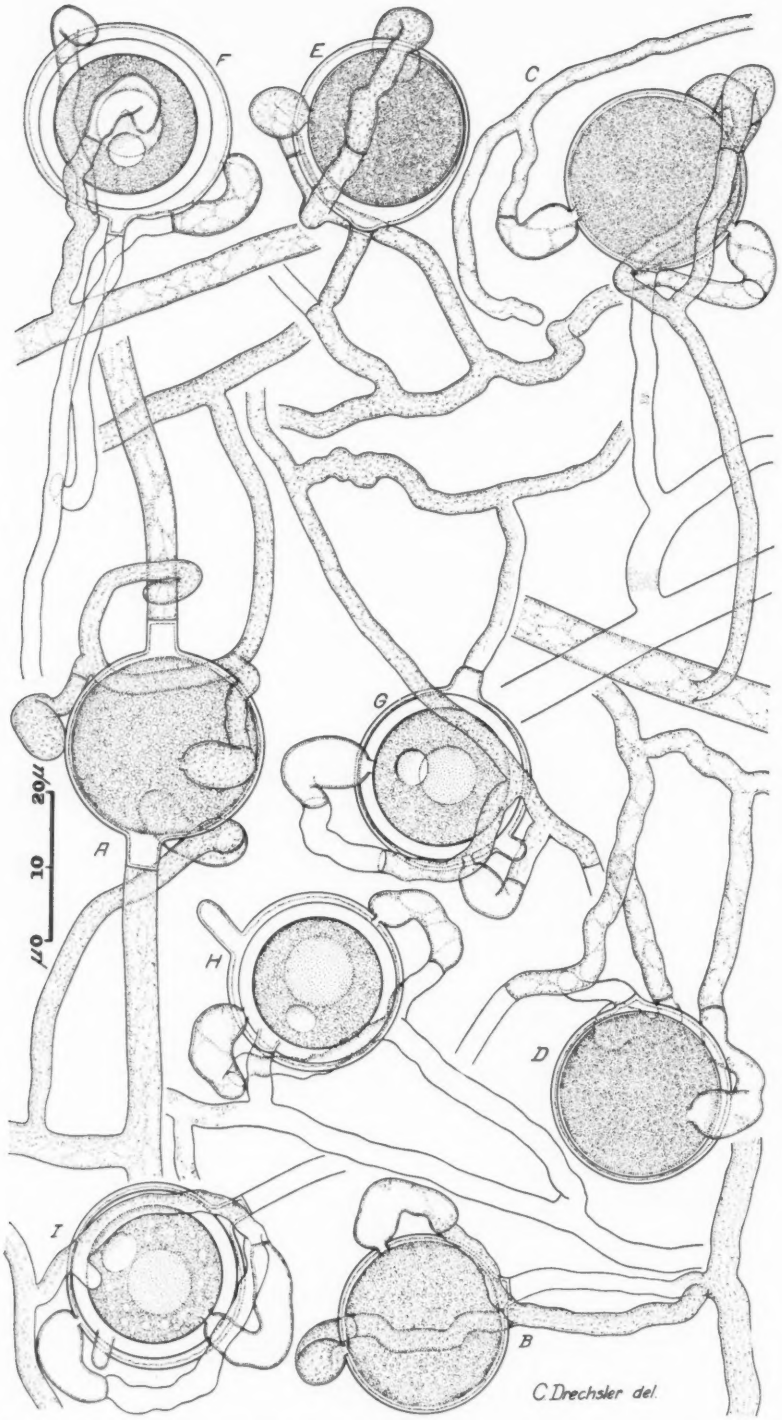

FIG. 1. Sexual apparatus of a Puerto Rican strain of *Pythium graminicolum* developed in a maize-meal agar plate culture, and drawn with the aid of the camera lucida. ×1000.

FIG. 2. Sexual apparatus of the type culture of *Pythium arrhenomanes* on maize-meal agar; drawn with the aid of the camera lucida. ×1000.

A conspicuously different relationship of parts is evident in figure 2, representing sexual apparatus of the type culture of *Pythium arrhenomanes* isolated from diseased roots of maize (*Zea mays* L.) by Helen Johann and employed in investigations reported by Johann, Holbert, and Dickson (7, 8). The mycelial connection between oogonium and antheridium here is much more distant than in *P. graminicolum;* usually, indeed, being too distant to be traced in the tangle of rather irregularly disposed filaments that make up the somewhat characteristic growth in agar plate cultures. In especially favorable material the continuity of hyphal elements between the male and the female organs can occasionally be seen, and, as in homothallic forms generally, such continuity is necessarily always present when a single mycelium occupies a tract of substratum. Rands and Dopp have well pointed out that the type culture produces antheridia more abundantly than most strains of the species. Yet the similarity in origin and arrangement of the male organs is recognizable without difficulty in strains producing these structures in more moderate quantity; as, for example, in the strain represented in figure 3, which was isolated from diseased maize rootlets submitted by W. D. Valleau as being typical of rootrot described from Kentucky by Valleau, Karraker, and Johnson (13). Figures 2 and 3 were drawn in 1927, and may, therefore, also be assumed to have been prepared in the absence of any possible prejudice bearing on the taxonomic issue under discussion.

Associated with the differences in arrangement of sexual apparatus are other differences, which, if less easily expressed in words or in drawings, are often experienced vividly enough when *Pythium graminicolum* and *P. arrhenomanes* are grown side by side on artificial substrata. On agar media containing maize-meal decoction with some of the finer maize-meal sediment, *P. graminicolum* develops a mycelium that in due course usually gives rise to oogonia and antheridia. For the most part each oogonium develops a normal oospore, which at maturity consists of a fairly thick wall, a parietal layer of granular protoplasm, a subspherical or ellipsoidal refringent body imbedded in the parietal lawer, and a central vacuole-like reserve globule (Fig. 1, F–I). The oogonial wall, the membrane of each antheridium, and the wall of each supporting hyphal element are so substantial that, after maturity, they, for the most part, long retain their respective original shapes without much alteration from collapse, and long remain very clearly visible to microscopic inspection. Asexual reproduction likewise is easily induced. When pieces of an agar plate culture with vigorous vegetative mycelium are appropriately irrigated, lobulate sporangial complexes are developed that freely give rise to zoospores. Although some strains are more refractory than others, the species, on the whole, readily reveals both of its reproductive stages, which, because of the continuing visibility of the sexual apparatus, are easy to study and to delineate.

FIG. 3. Sexual apparatus of a Kentucky strain of *Pythium arrhenomanes* developed in a maize-meal agar plate culture, and drawn with the aid of the camera lucida. ×1000.

Pythium arrhenomanes, on the other hand, offers many exasperating difficulties. On ordinary maize-meal agar vegetative growth is profuse, and with many strains sex organs as well as massive lobulate sporangial complexes are soon produced in quantity. But the oogonia, together with the antheridia supplying them, almost without exception undergo degeneration, their contents either breaking down or migrating into hyphal outgrowths. Through the use of special media, like those devised by Johann (6) and by Rands and Dopp (10), the production of sexual apparatus can be stimulated, and its degeneration reduced; some strains, as, for example, the one represented in the type culture, then yielding oospores of normal structure in more than half of the numerous oogonia. Yet, even after normal development has been obtained, the frequently haphazard and rather intricate disposition of the rangy antheridial filaments is not always readily made out. Moreover, after fertilization has been completed, the antheridia and the branches supporting them become increasingly difficult to see; with the result that on maturity of the oospore they have become for the most part nearly indiscernible. The hypha bearing the oogonium similarly becomes less clearly visible, and even the oogonial wall will often lose much of its optical distinctness.

Though massive lobulate complexes are often formed abundantly when *Pythium arrhenomanes* is cultivated on maize-meal agar, these structures, on being irrigated in a manner successful for zoospore production in many congeneric forms, exhaust themselves in a promiscuous proliferation of aerial or submerged filamentous outgrowths, without giving any sign of normal sporangial development. Zoospore formation, it is true, has been observed a few times on irrigating pieces of Lima bean decoction agar occupied by vigorously growing mycelium; the output in all such instances being, however, very meager in comparison with that ensuing when freshly invaded maize roots are placed in water. The strong tendency toward degeneration of reproductive bodies, noticeable on most artificial media in common use, combined with the intricacy of the sexual apparatus and the early evanescence especially of the male parts, has undoubtedly been responsible in no small degree for the unimpressive descriptive treatment that long permitted the species to remain in obscurity.

A closer approach to the morphology and cultural traits of *Pythium arrhenomanes* than is seen in *P. graminicolum,* or, for that matter, in any other form known to me, is evident in a species I have described elsewhere (5) as *P. myriotylum.* The extraordinary haustorial development associated with the frequently aerial parasitism characteristic of this species, and the less pronounced inflation of its sporangial complexes, which, after developing on artificial media, rather readily give rise to zoospores, should suffice, nevertheless, to distinguish it from *P. arrhenomanes. P. myriotylum* has

not hitherto been recognized in any collections of ultures derived from diseased roots of graminaceous plants; and in a soil at all compact, would seem incapable of operating at a sufficient depth to attack plant parts far under ground. Its remarkable parallelism in parasitic habit and host range to *P. butleri* may be expected to make for its orientation with reference to that species of much different morphology, rather than for orientation with reference to the fundamentally more similar and apparently more closely related graminicolous form.

SUMMARY

Though *Pythium graminicolum* and *P. arrhenomanes* are both parasitic on sugar cane, being associated abundantly with root rots of this host in the southern United States, they represent separate species rather than a single species, as has recently been averred. A close mycelial connection between oogonium and antheridium, very frequent in *P. graminicolum,* is rare in *P. arrhenomanes;* and in parallel cultures the sturdy, more substantial membranous parts of the sexual apparatus of the former species remain clearly discernible long after the evanescent antheridial envelopes and supporting branches of the latter have become nearly or wholly invisible.

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THE OCCURRENCE OF GIBBERELLA FUJIKUROI VAR. SUBGLUTINANS IN THE UNITED STATES[1]

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INTRODUCTION

Specimens of perithecia that superficially appeared like those of *Gibberella saubinetii* (Mont.) Sacc. were found on old corn stalks in New Jersey and Ohio. Microscopic examination of the fruiting bodies indicated that they were not those of *G. saubinetii,* since the asci were long and relatively narrow, and bore straight, 1-septate ascospores. A few of the asci contained 4 or 6 ascospores instead of the usual 8. These observations suggested that the fungus might be *Gibberella fujikuroi* (Saw.) Wr.[2] On culturing a number of single ascospores, however, it was found that microconidia were never produced in chains as in *Fusarium moniliforme* Sheld., the imperfect stage of *G. fujikuroi.* Further observations strongly indicated that the fungus was *G. fujikuroi* (Saw.) Wr. var. *subglutinans* Edwards.

Since *Gibberella fujikori* var. *subglutinans* Wr. and Rg. and its conidial stage, *Fusarium moniliforme* Sheld. var. *subglutinans* Wr. and Rg. have never been reported in this country, and since this variety and its species, *G. fujikuroi,* are very similar morphologically, a comparison of the two together with their respective imperfect forms seemed advisable in order to confirm the identity of the fungus in question.[3]

G. fujikuroi var. *subglutinans* was first reported by Edwards (1) in New South Wales, Australia, in 1933. He found the perithecia on old corn stalks and demonstrated the pathogenicity of the conidial stage on the seedlings, stalks, and ears of corn, *Zea mays* L. The imperfect, or conidial stage, *Fusarium moniliforme* var. *subglutinans,* has been reported on a number of hosts including banana, *Musa sapientum* L. (6), pineapple, *Ananas sativus* Schult. (7), manila hemp, *Musa textilus* Nee (6), sugar cane, *Saccharum officinarum* L. (3), and wheat, *Triticum sativum* Lam. (2). Recently the fungus has been reported from New Zealand as the cause of pink cob-rot of corn (4).

[1] Published at the expense of The Rockefeller Institute for Medical Research, Princeton, N. J., out of the order determined by the date of receipt of the manuscript. This practice in nowise delays the publication of manuscripts printed at the expense of The American Phytopathological Society or other agency.

[2] In a recent treatise on the genus Fusarium (9), Wollenweber and Reinking have used the binomial *G. fujikuroi* (Saw.) Wr. in preference to its synonym *G. moniliformis* (Sh.) Winel.

[3] The writer is indebted to C. D. Sherbakoff for substantiating the identity of this fungus.

The ascigerous stage of the fungus is closely related morphologically to *Gibberella fujikuroi,* but differs from the latter in that 8-spore asci are more common than the 4- to 6-spore asci, and the 2- to 3-septate ascospores are fewer in number. In addition, the ascospores are more blunt and are short-elliptical in shape. The conidial stage, *Fusarium moniliforme* var. *subglutinans,* is primarily differentiated from the type form, *F. moniliforme* Sheld., of the section Liseola in the genus Fusarium Link, in that the microconidia are not borne in chains.

EXPERIMENTAL

It is well known that the Fusaria, as a group, are extremely variable, and that this variation may be expressed physiologically, morphologically, and in cultural characters. In spite of this variability, it was believed that certain differences, especially of the more stable characters, might be brought out between the two forms.

Morphology. The morphological characteristics of the ascigerous stage of *Gibberella fujikuroi* var. *subglutinans* were studied as the fungus occurred on dead corn stalks. The conidial stage was studied while growing on Lima bean agar in pure culture. All cultures were of single ascosporic origin.

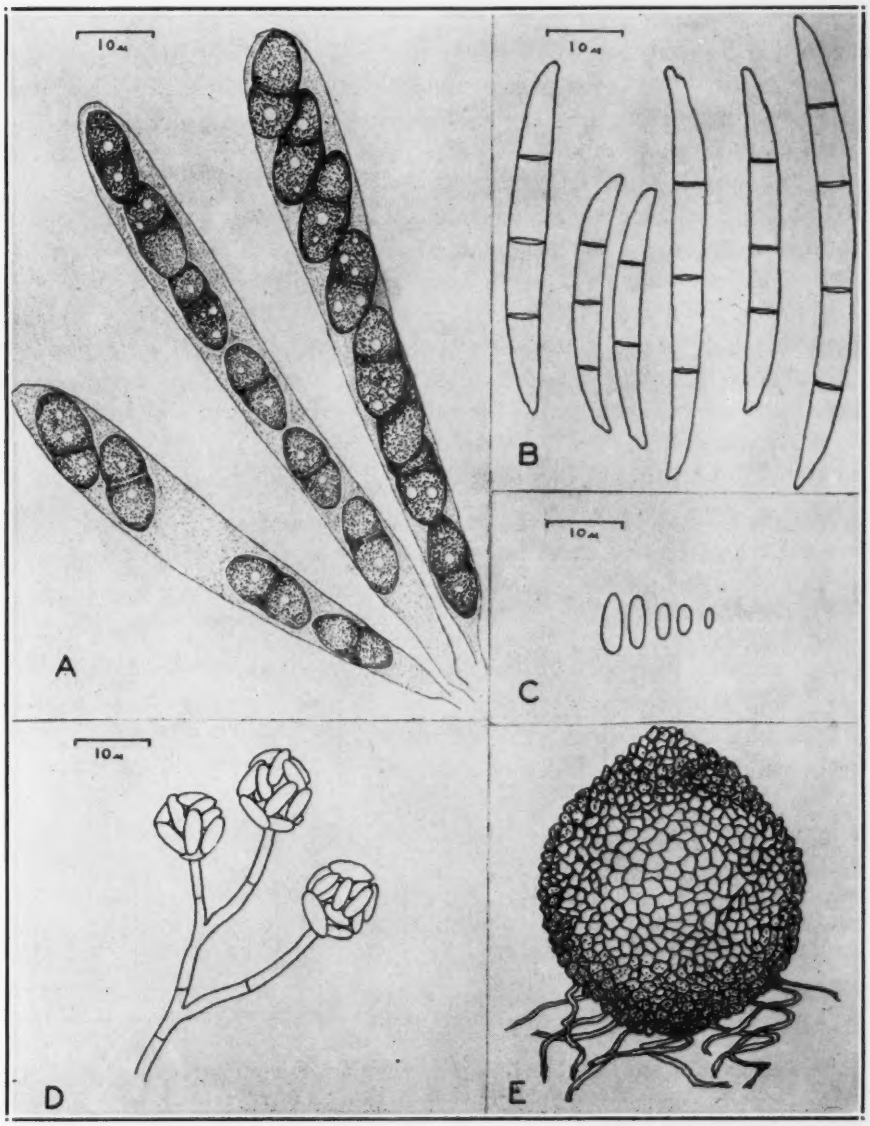
The following are measurements of conidia from a 15-day-old colony grown on Lima bean agar at room temperature (22–24° C.). The average spore size in each of the following groups is based on the measurements of 100 conidia selected at random:

0—septate	$10.1 \times 2.9\ \mu$	($7.4–14.9 \times 2.4–3.3\ \mu$)
1—septate	$20.0 \times 3.3\ \mu$	($14.9–26.5 \times 2.4–3.7\ \mu$)
2—septate	$25.1 \times 3.7\ \mu$	($21.5–33.2 \times 3.3–4.5\ \mu$)
3—septate	$40.0 \times 3.8\ \mu$	($29.8–51.4 \times 3.4–4.5\ \mu$)
4—septate	$51.6 \times 3.9\ \mu$	($41.5–59.7 \times 3.4–4.9\ \mu$)
5—septate	$54.6 \times 3.9\ \mu$	($48.1–59.7 \times 3.5–4.9\ \mu$)

The microconidia are borne on simple or branched conidiophores, usually in false heads (Fig. 1, D), or singly, but never in chains on any type of substrate. Macroconidia (Fig. 1, B), which are borne on the aerial mycelium, in sporodochia, or pionnotes, are 1- to 5-septate, the 3-septate spores being most abundant.

The ascospores (Fig. 1, A) are straight, slightly constricted at the septa, and short-elliptical in shape. They are predominantly 1-septate, although a few 2-septate spores have been observed. The 1-septate spores measured $12.1–17.0 \times 4.5–7.0\ \mu$, averaging $14.8 \times 5.6\ \mu$.

The asci are relatively long, narrow, and subclavate in shape (Fig. 1, A). Usually 8 ascospores are found within each ascus; 4- and 6-spore asci are only occasionally found. The asci measured $75–100 \times 10–16\ \mu$.



Photographed by J. A. Carlile

FIG. 1. A. 4-, 6- and 8-spore asci. B. Macroconidia. C. Microconidia. D. Micronidia showing the false heads in which they are borne. E. Perithecium. About × 120.

The perithecia, which are practically indistinguishable from those of *Gibberella saubinetii,* measured 270–350 × 240–300 μ (Fig. 1, E). They are borne superficially on corn stalks and occur either scattered or in groups.

Growth on Various Media. Eight single ascosporic cultures (B–1 to B–8), all of which were derived from the same ascus, together with 2 isolates

of *Fusarium moniliforme*[4] (E–1 and Y–29), were grown on 8 different agar media at room temperature. Each culture was seeded in triplicate on each of the 8 media. The pieces of mycelium used to seed the plates were of uniform size and cut from the marginal growth of young colonies that had been grown on the same medium. The diameters of the colonies were measured every 24 hours for a period of 7 days.

In table 1 are shown the diameters of the colonies, at the end of the 7th day, grown on the 8 media. Isolates B–1 and B–8 generally gave the most rapidly-growing colonies of *Fusarium moniliforme* var. *subglutinans* on all media. The two cultures of *F. moniliforme* grew more slowly on most media than did those of *F. moniliforme* var. *subglutinans;* this was especially noticeable on potato-dextrose agar. On Coon's medium agar, however, a marked difference in the general trend of growth rate was observed. Here the two cultures of *F. moniliforme* showed a more rapid rate of growth than did any of the cultures of *F. moniliforme* var. *subglutinans.*

TABLE 1.—*Colony diameters in millimeters of 7-day-old cultures of Fusarium moniliforme var. subglutinans and F. moniliforme grown at room temperature on 8 agar media*

Agar media	Colony diameters in millimeters									
	F. moniliforme var. subglutinans								F. moniliforme	
	B–1	B–2	B–3	B–4	B–5	B–6	B–7	B–8	E–1	Y–29
Malt	78	70	72	78	70	72	70	81	73	65
Prune	76	73	72	75	75	70	71	75	75	70
Potato-dextrose	90+[a]	90+	90+	90+	90+	90+	85	90+	75	70
Oatmeal	78	73	75	75	74	75	74	76	72	65
Cornmeal	82	76	76	76	76	73	72	80	65	65
Lima bean	81	74	75	78	78	68	68	85	68	65
Richard's	76	71	69	73	71	74	73	80	73	73
Coon's	67	65	67	67	65	67	65	67	74	72

[a] The + sign indicates that the colonies had grown over the entire surface of the agar.

The colonies listed in table 1, together with single cultures of each of the isolates growing on steam-sterilized rice, were held until 15 days of age, when observations on color production were made. A comparison of the stromatic color between *Fusarium moniliforme* var. *subglutinans* and *F. moniliforme* growing on different media is given in table 2. No appreciable differences were observed in color production between the isolates of *F. moniliforme* var. *subglutinans,* or between the 2 isolates of *F. moniliforme.*

[4] The cultures of *F. moniliforme* were furnished through the kindness of Miss Helen Johann of the United States Department of Agriculture.

TABLE 2.—*Comparison of the stromatic color of Fusarium moniliforme var. subglutinans and F. moniliforme grown on different media*

Differential media	Color of stroma on various media	
	F. moniliforme var. subglutinans	F. moniliforme
Malt agar	Purplish red[a]	Veronia purple
Prune "	Transparent white	Transparent white
Potato-dextrose "	Blackish-red-purple	Vinaceous purple
Oatmeal "	White	Russet vinaceous
Cornmeal "	Transparent white	Transparent white
Lima bean "	" "	" "
Richard's "	Cream buff	Apricot yellow
Coon's "	White	White
Steamed rice	Dahlia carmine	Cream color

[a] Colors based on Ridgeway's standards—Ridgeway, R. Color standards and nomenclature. 43 pp. Washington, D. C. 1912.

The most striking difference in color between the two species was brought out on steam-sterilized rice, a critical medium for color diagnosis (10).

Growth at Different Temperatures. One culture of *Fusarium moniliforme* var. *subglutinans*, B–6, and 2 cultures of *F. moniliforme* were grown grown on potato-dextrose agar at 5, 10, 15, 20, 25, 30, 35 and 40° C. The plates were seeded in triplicate with uniform amounts of mycelium from 7-day-old colonies grown at the same temperatures. In cases of the extreme temperatures, where certain cultures failed to grow, the mycelium used to seed the plates was taken from a colony grown at the next closest temperature. The diameters of the colonies were measured every 24 hours.

In table 3 are given the diameters of the colonies in millimeters at each temperature at the end of 6 days. The isolate of *Fusarium moniliforme* var.

TABLE 3.—*Colony diameters at the end of the 6th day of Fusarium moniliforme and F. moniliforme var. subglutinans grown on potato-dextrose agar at 8 different temperatures*

Isolate	Colony diameters (in mm.) at different temperatures							
	5°	10°	15°	20°	25°	30°	35°	40°
E–1, F. moniliforme	7	20	37	55	85	90	31	0
Y–29, F. moniliforme ...	0[a]	17	34	38	72	75	16	0
B–6, F. moniliforme var. subglutinans	11	25	41	57	82	77	8	0

[a] The slight growth occurring here was not measurable.

subglutinans, B–6, showed a markedly lower growth-temperature range, with a minimum below 5° C., an optimum at about 25° C., and a maximum between 35° and 40° C. The isolates of *F. moniliforme* showed less growth below 20° C. and a somewhat higher optimum of about 30° C. Although the maximum temperature for growth is approximately the same for *F. moniliforme* var. *subglutinans* and *F. moniliforme*, the latter is able to grow considerably better at 35° C.

Pathogenicity. In order to determine the pathogenicity of the fungus, kernels of yellow dent corn were planted in rich compost soil that had been infested with cornmeal-sand cultures of the fungus at the rate of 4 parts of soil to 1 part of culture. Control plantings were made in soil mixed in the same proportion with sterile cornmeal-sand medium. The experiment was carried out in a greenhouse where a soil temperature of about 22° C. was maintained.

When the control seedlings had reached the 3-leaf stage, 10 days after planting, all seedlings were removed from the soil, washed clean, and examined for disease. Over 80 per cent of seedlings in the inoculated lots showed varying degrees of blighting, while those of the control plantings showed less than 10 per cent disease. From the inoculated seedlings *Fusarium moniliforme* var. *subglutinans* was readily reisolated, whereas the diseased seedlings from control plantings gave rise to *Penicillium* sp., *Aspergillus* sp., and an undertermined *Fusarium* sp.

In general, the symptoms of corn seedling blight brought about by infection with this fungus were very similar to those caused by *Fusarium moniliforme* (8). A marked delay in emergence, followed by a retardation in growth, was the first indication of infection. The root systems of inoculated kernels were reduced in extent (Fig. 2) and marked with numerous reddish-brown lesions. Lesions of varying size were found centering around points where primary and adventitious roots break through coleorhiza and mesocotyl, respectively.

Sexuality. To determine if sex groups existed within the variety, a number of experiments were conducted in which single cultures, and cultures paired in all possible combinations, were grown on different substrates.

In one experiment, 8 cultures, each of single ascosporic origin and derived from a single ascus, were grown individually and in all possible pairings on plants of potato-dextrose agar. At the end of 2 months, at which time the experiment was discontinued, there was no indication of perithecia in any of the cultures.

A second experiment was conducted in which 9 cultures, 8 single and 1 a composite of all 8, were grown on plates of ground whole-oat agar. After a lapse of 2 months no perithecia were observed in the cultures.

FIG. 2. The 3 seedlings at the left are healthy. The 7 seedlings at the right show all degrees of infection with *Fusarium moniliforme* var. *subglutinans*.

In a third experiment, 9 cultures, 8 single and 1 a composite of all 8, were grown on sterilized whole oats in 250-cc. Erlenmeyer flasks. After a period of 1 week these cultures were placed in separate earthenware pots containing sand, covered with cheesecloth, and removed to the greenhouse. The pots were set far apart and precautions were taken to prevent contamination of one culture by another. Within 3 weeks after placing the cultures in the greenhouse, during which time they were watered daily, mature perithecia

had formed in cultures B–5 and B–8, and in the composite culture of all 8 isolates. A number of the cultures produced an abundance of perithecia, but the latter remained small and never reached maturity. When the experiment was repeated, essentially the same results were obtained, with the exception that a longer time (5 weeks) elapsed before mature perithecia were observed in culture B–5.

These experiments indicate that the fungus is homothallic and that some cultures, originally derived from single ascospores, are able to produce mature perithecia if given the proper environmental conditions. Other cultures of similar origin, but perhaps different physiologically, are unable to develop mature perithecia under the same conditions.

DISCUSSION

Fusarium moniliforme var. *subglutinans* and *F. moniliforme* are the only members of the section Liseola of the genus Fusarium in which an ascigerous stage is known. Furthermore, their perfect stages, namely, *Gibberella fujikuroi* var. *subglutinans* and *G. fujikuroi*, are the only known members of the genus Gibberella in which 1-septate ascospores are predominant.

In the present studies the measurement of the spore forms, together with other diagnostic characters, correspond very closely to those given by Wollenweber and Reinking (9). In view of the above facts, along with differentiating characters brought out in the experiments reported in this paper, there appears to be little doubt as to the identity of this fungus.

It is probable that a wider distribution of the fungus will be found in this country than indicated in the present paper. The question as to whether it is endemic to the United States and has been overlooked or mistaken for *Gibberella fujikuroi*, or has recently been introduced into this country, is difficult to answer. In spite of the possibility of confusion between the species and its variety, *subglutinans*, the most plausible explanation for the occurrence of the variety in the United States seems to be that of recent introduction.

SUMMARY

The occurrence of *Gibberella fujikuroi* var. *subglutinans* in the United States is reported and descriptions of its morphology and cultural characters are given.

The conidial stage of the fungus could be differentiated from *Fusarium moniliforme* by its faster growth rate and more intense coloration on different media, particularly on steamed rice.

The minimum and optimum temperatures for growth of the varietal form were somewhat lower than those of the type species, *F. moniliforme*.

Certain cultures of single ascosporic origin were shown to be capable of producing mature perithecia, indicating that the fungus is homothallic.

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CHEMICAL CONTROL OF HARMFUL FUNGI DURING STRATIFICATION AND GERMINATION OF SEEDS OF RIBES ROEZLI

CLARENCE R. QUICK

(Accepted for publication September 3, 1935)

INTRODUCTION

The research results here reported were obtained under the authority of the Division of Blister Rust Control, while it was yet in the Bureau of Plant Industry, U. S. Department of Agriculture.[1]

In carrying out a program of research[2] on the physiology and morphology of plants of the genus *Ribes* L., currants and gooseberries, it has been necessary to grow large numbers of seedlings for experimental use. The seeds of most Ribes species germinate most satisfactorily after several months of moist stratification at low temperatures. Preliminary work on the routine propagation of Ribes plants showed that damping-off fungi invariably caused a high seedling mortality during the extended period of stratification and germination. The experiments summarized in this paper represent a study of practical methods for controlling damping-off fungi in Ribes seed cultures during stratification and germination.

EXPERIMENTAL PROCEDURE

The seeds of *Ribes roezli* Regel used in the experiments were collected in the fall of 1930 at an elevation of about 6,300 feet, in the Stanislaus National Forest of California. They were stored as air-dry seeds at room temperature for 1 year, and air-dry at 5° C. for an additional year. The seeds were in excellent condition when planted in the cultures at the beginning of the tests. Two series of cultures were run.

In the first series of cultures, 65 paraffined paper cups were filled to within ½ inch of the top with a mixture of equal parts river sand, fine forest loam, and Sphagnum moss which had been passed through a 12-mesh sieve. The cups held about 105 ml. of soil, had a soil surface of about 24.5 sq. cm. (3.8 sq. in.), and required about 60 ml. of water or chemical solution to saturate the propagating medium. The sand had been used in previous experiments and was known to be heavily infested with damping-off fungi. Sixteen reagent chemicals[3] were selected for the tests, and were applied

[1] Effective December 1, 1933, the Blister Rust Control work was transferred to the Bureau of Entomology and Plant Quarantine.

[2] This work, part of an investigative program on the chemical eradiction of Ribes, has been made possible through the active cooperation of the Department of Botany and the College of Agriculture of the University of California at Berkeley.

[3] By the term "reagent chemicals" the writer signifies those chemicals commonly kept in stock in a laboratory.

in the manner best adapted to their chemical properties. Thus, liquid chemicals were diluted with water and used to saturate the soil of the culture; and solids were applied (a) in aqueous solution, (b) as a dry powder, or (c) suspended in water. All treatments, except those in which the seeds were dusted with dry powder, were made immediately after the seeds were planted in the cultures. Each culture consisted of 40 seeds of *Ribes roezli* planted at a depth of about ½ cm.

The 65 cultures[4] were placed in 4 small flats, covered with paraffined paper and subjected to a period of 3½ months of moist stratification at 2½° C. In April, 1933, the flats were removed to a greenhouse for germination test.

A second set of 35 cultures[5] was started in October 1933, using seeds of the same collection. The same type of propagating medium and the same period of stratification at 2½° C. were employed. To reduce crowding of the seedlings, larger paraffined cups were used than in the first set of cultures. These larger containers held about 120 ml. of soil, had a soil surface of about 42 sq. cm. (6.5 sq. in.), and required about 75 ml. of solution to saturate the propagating medium.

The most satisfactory seedling production was obtained by the use of copper oxalate, basic copper carbonate, formaldehyde, and nitric acid. As compared with an average of 5 per cent of seedlings satisfactory for propa-

[4] A complete list of cultures of the first set is given below. Controls 3. Seeds dusted with basic cupric carbonate powder, and with anhydrous cupric sulphate. Soil treated with flowers of sulphur at rates of 2, 4, 8, and 16 grams per 946 ml. (1 qt.) of soil and with chlorinated lime at rates of 2, 4, and 8 grams per 946 ml. soil. Cultures treated with basic cupric carbonate powder at rates of ½, 1 2, 4, and 8 grams per 929 sq. cm. (1 sq. ft.) of surface; with cupric oxalate at the same rates; and with mercurous chloride at rates 0.05, 0.10, 0.15, and 0.20 grams per 929 sq. cm. Glacial acetic acid was applied to cultures in dilutions (by volume) of 1:500, and 1:1000. Formaldehyde, 40 per cent, 1:400, 1:600, and 1:800. Sulphuric acid, 1:250, 1:500, and 1:1000. Nitric acid, 1:250, 1:500, and 1:1000. Sodium hypochlorite, 5 per cent solution, 1:125, 1:250, 1:500, and 1:1000. Hydrogen peroxide, 3 per cent, 1:5, 1:11, and 1:23. Mercuric chloride was dissolved in water and applied to cultures in dilutions (by weight) of 1:500, 1:1000, 1:2000, and 1:4000. Aluminum sulphate (hydrated) 1:62.5, 1:125, 1:250, 1:500, and 1:1000. Cupric sulphate (hydrated) 1:250, 1:500, 1:1000, and 1:2000. Sodium chlorate, 1:500, 1:1000, 1:2000, 1:4000, and 1:8000. Boric acid, 1:500, 1:1000, and 1:2000.

[5] The complete list of cultures of the second set follows. Controls 2. Seeds were dusted with basic cupric carbonate. Cultures were treated with basic cupric carbonate at the rates of 2, 3, 4, 6, and 8 grams per 929 sq. cm. (1 sq. ft.); and with cupric oxalate at rates of 3, 6, 9, and 12 grams per 929 sq. cm. Glacial acetic acid was applied to cultures in dilutions (by volume) of 1:500, 1:1000, and 1:1500. Formaldehyde, 40 per cent, 1:750, 1:1000, and 1:1250. Hydrogen peroxide, 3 per cent, 1:2. Nitric acid, specific gravity 1.42, 1:125, 1:200, 1:250, 1:350, and 1:500. Sulphuric acid, specific gravity 1.84, 1:500, 1:1000, and 1:2000. Sodium hypochlorite, 5 per cent solution, 1:1000 and 1:2000. Aluminum sulphate (hydrated) was dissolved in water (by weight) and applied in dilutions of 1:15, 1:30, and 1:60. Copper sulphate (hydrated) 1:125, 1:250, and 1:500.

gation in the untreated cultures at the end of the test, the copper oxalate cultures averaged 42 per cent, the formeldehyde 34 per cent, the nitric acid 29 per cent, and the copper carbonate 24 per cent. Table 1 presents for comparison the results of some of the more satisfactory tests.

TABLE 1.—*Percentage production of seedings in some of the more satisfactory cultures*

Chemical used	Dilution of substance with water	Grams per 929 sq. cm. (1 sq. ft.) soil surface	First set cultures		Second set cultures	
			Satisfactory seedlings in culture at end of test	Total seedlings produced	Satisfactory seedlings in culture at end of test	Total seedlings produced
Water (controls)	.....	.....	3	11	6	39
Copper carbonate						
$CuCO_3 \cdot Cu(OH)_2$, C. P.	.....	2	28	70	.....	.....
do	.....	3	.....	.....	6	44
do	.....	4	38	78	.....	.....
do	.....	6	.....	.....	14	80
do	.....	8	23	100	36	56
Copper oxalate						
$Cu(COO)_2 \frac{1}{2} H_2O$, C. P.	.....	3	.....	.....	26	56
do	.....	4	18	63	.....	.....
do	.....	6	.....	.....	64	70
do	.....	8	50	98	.....	.....
do	.....	9	.....	.....	56	64
do	.....	12	.....	.....	40	74
Formaldehyde						
HCHO, 40%, U. S. P.	1:600	.....	20	40	.....	.....
do	1:750	.....	.....	.....	74	98
do	1:800	.....	30	58	.....	.....
do	1:1250	.....	.....	.....	12	92
Nitric acid						
HNO_3, 70%, C. P.	1:200	.....	.....	.....	22	70
do	1:250	.....	58	80	10	90
do	1:500	.....	50	70	4	98

Finely powdered cupric oxalate gave good protection to seeds and seedlings when applied to cultures at rates of 6 to 10 grams of chemical per 929 sq. cm. (1 sq. ft.) of soil surface. Its protection lasted throughout the $3\frac{1}{2}$ months of moist stratification at $2\frac{1}{2}°$ C., and a subsequent 6 weeks' germination test in the greenhouse. Finely powered basic cupric carbonate was found satisfactory, but slightly less effective than cupric oxalate. The best rates of application were 4 to 8 grams per 929 sq. cm. The most convenient methods of applying the insoluble powders were: (1) to distribute the dry

powder evenly over the surface of the culture and then to wet down with a florist's syringe, or (2) to suspend the powder in water and to apply to the surface of the culture. Since the completion of the experimental work, copper oxalate and copper carbonate have been used repeatedly and successfully for the control of damping-off fungi in cultures of a wide variety of Ribes seeds. The use of these two compounds on seed cultures of other plants, such as snapdragon, petunia, etc., has consistently protected the seedlings from damage by fungi.

Dilute nitric acid and formaldehyde protected the seeds fairly well during stratification (the former causing a marked acceleration of seedling growth), but, presumably because of high reactivity and consequent rapid disappearance, gave less sure protection than the relatively insoluble copper compounds. In the nitric acid and formaldehyde series, best results were secured with a solution of 1 part of 70 per cent nitric acid to 250 parts of water and with 1 part of commercial 40 per cent formaldehyde to 750 parts of water.

FEEDING HABITS OF THE NEMATODES APHELENCHOIDES PARIETINUS AND APHELENCHUS AVENAE

J. R. Christie and C. H. Arndt

(Accepted for publication Sept. 23, 1935)

It appears to be the prevalent opinion of workers in the field of free-living and plant-parasitic nematodes that *Aphelenchoides parietinus* (Bastian, 1865) Steiner, 1932 and *Aphelenchus avenae* Bastian, 1865 are saprophytes. Thorne[1] refers to *Aphelenchus avenae* as a saprophytic species and Goodey[2] regards both *Aphelenchoides parietinus* and *Aphelenchus avenae* as "mainly saprophytic in habit." The latter investigator cites this saprophytic habitat as evidence possibly indicating that the stylet of plant-infesting members of the Anguillulinidae may not necessarily be "a puncturing or offensive organ." In this connection Goodey goes on to say, "Again, there seem to be no records of direct observations of the stylet actually functioning as a puncturing organ. Admitting that such evidence may be extremely difficult to obtain, it is surely not without significance that no one has, so far, produced it." This being the case, observations by the writers on the habits of *Aphelenchoides parietinus* and *Aphelenchus avenae* appear worthy of record.

Both parietinus and avenae can be reared on agar plates. A cornmeal nutrient agar on which the fungus *Neurospora sitophila* is growing has been found satisfactory and was used by the writers in their experimental work with these nemas. While attempting to determine the most suitable culture medium it was noted that avenae and parietinus developed and reproduced only when the plate was contaminated with a fungus. It was further noted that the nemas frequently gather near the periphery of a fungus colony. By using a comparatively transparent agar and pouring thin plates it is possible to place the preparation on the stage of a microscope and observe the specimens under a magnification of $\times 150$. An individual that has penetrated the agar and is located near the bottom of the Petri dish is more satisfactory for study, as the agar retards movement and the preparation can be inverted and the nema viewed through the bottom of the dish. Under these conditions the feeding habits of these nemas can be distinctly seen. After the head is placed against a hypha there is a brief but rapid backward and forward movement of the stylet followed immediately by an equally rapid muscular movement of the bulb. Granules and other cellular inclusions in the hypha can be seen moving towards the point where the

[1] Thorne, Gerald. The nematode *Neotylenchus abulbosus* Steiner (Anguillulinidae) as a parasite of sugar-beets. Helminthol. Soc. Wash. Proc. **2**: 46. 1935.

[2] Goodey, T. The pathology and etiology of plant lesions caused by parasitic nematodes. 34 pp. Imp. Bur. Agr. Parasitol., St. Albans, Eng. 1935.

stylet is inserted. The stylet did not remain appreciably protruded while the contents of the hypha were being removed. These observations apply both to parietinus and avenae and leave little doubt as to the source of food of these

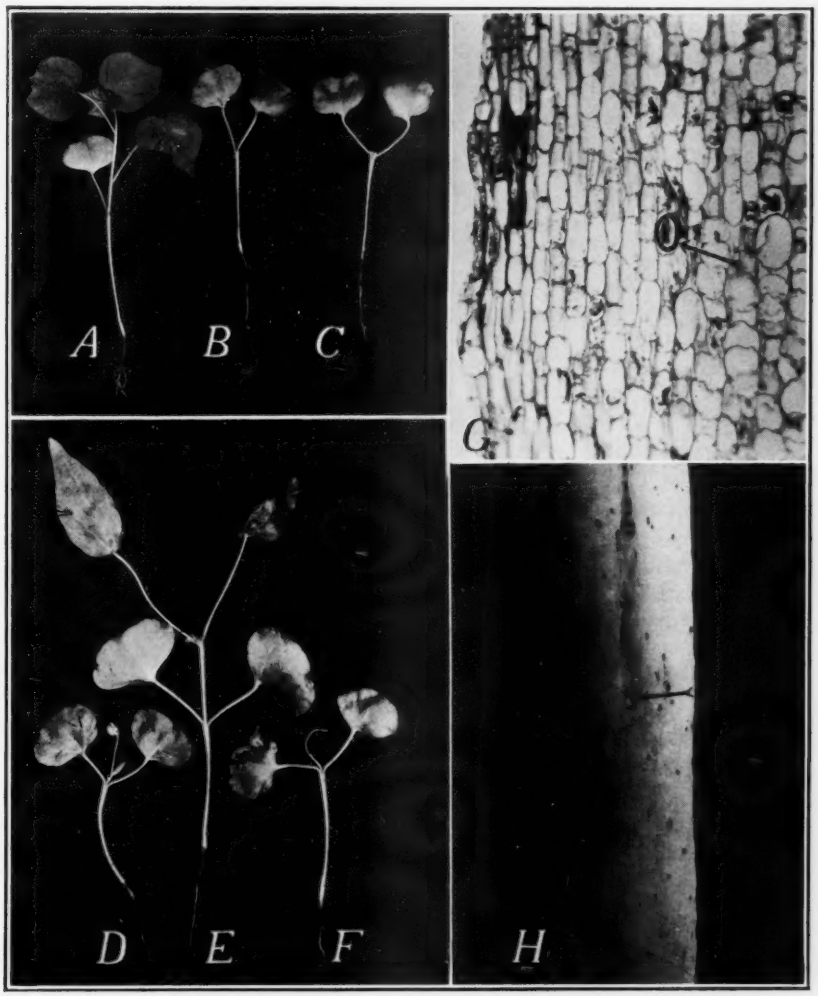

FIG. 1. Cotton seedlings attacked by the nematode *Aphelenchoides parietinus*. A. Normal seedling. B to F. Seedlings the terminal buds of which are infested with *A. parietinus*. These seedlings, grown at a constant soil temperature of 21° to 22° C., required 7 to 9 days for germination. G. Longitudinal section through cotton hypocotyl showing specimen of *A. parietinus* within cortical cell and surrounded by apparently healthy tissue. The diseased tissues of a shallow lesion show on the upper left side of the photograph. H. Cotton hypocotyl showing small pit presumably caused by *A. parietinus* and from which three living specimens of this nema were removed.

nemas when they are growing on culture media. In nature parietinus and avenae are frequently found associated with decaying organic matter. However, this is likewise a favored environment for many fungi and it seems probable that the nemas are attracted by such fungal growth.

There is considerable evidence that fungi are not the only source of food. Both parietinus and avenae are frequently found associated with lesions on the underground parts of plants, especially on the hypocotyls of seedlings. Sometimes these nemas migrate beyond the diseased area, penetrating apparently healthy cortical tissue, and may be found coiled within cells (Fig. 1, G) or in intercellular spaces. In such instances it seems reasonable to suppose that they are utilizing the contents of these cells as food.

In connection with investigations regarding the cause of lesions on the hypocotyls of cotton seedlings the writers have carried on a series of greenhouse experiments growing the seedlings in soil experimentally infested with a species of nematode or a species of fungus or a combination of a nematode and a fungus. The soil was first steam-sterilized and the nematodes and fungi used for its subsequent inoculation were both culture-reared. This work is still in progress and complete results will be published later, but certain observations may appropriately be recorded in connection with the present discussion.

One experiment involved 25 lots of seedlings, 5 lots of which were grown in soil infested with parietinus. When the seedlings were removed and the roots washed it was noted that, in these 5 lots from parietinus-infested soils, the roots had a yellowish to brownish color, sufficiently pronounced to readily distinguish them from the other 20 lots. The explanation appeared to be that parietinus had been feeding on the epidermal cells of the roots.

In these and similar experiments with cotton seedlings, parietinus sometimes infested the terminal bud. This infestation undoubtedly took place before the seedling broke through the soil and was carried up as the plant grew. It occurred when germination took place at constant soil temperatures of 21° C. and below. It was not observed when the cotyledons emerged from the soil within 5 days after the time of planting. Infested plants are sometimes "blind," failing to produce secondary leaves (Fig. 1, B and C) or, if secondary leaves develop, they may be crinkled (Fig. 1, E) or small and distorted (Fig. 1, D and F) according to the degree of injury to the terminal bud. On dissecting the terminal buds of these abnormal plants from 1 to 10 specimens of parietinus per bud were found among the bases of the leaf primordia; never definitely internal to the epidermis. In such instances parietinus behaves very much like *Aphelenchoides fragariae* on strawberries.

Small pits frequently occur on the hypocotyls of cotton seedlings, which, on microscopical examination, are found to harbor specimens of parietinus

(Fig. 1, H). These pits are apparently of mechanical origin, and one feels justified in concluding that they are produced by the nemas. A previously reported experiment by the writers[3] furnishes further evidence that nemas can and do produce such pits. In this experiment nemas [mostly *Panagrolaimus* sp. (= *Cephalobus* of authors, in part)] were placed on or near the hypocotyl of a cotton seedling growing in sterile agar. Sixteen hours later 6 small pits had been produced in which nemas were found.

When cotton seedlings were grown at a constant soil temperature of 18 to 19° C. requiring 12 to 15 days for germination, there sometimes persisted on the cotyledons yellowish brown areas that did not turn green as normal tissue. Living specimens of parietinus were found in these areas. This occurred only on seedlings grown in soil inoculated with parietinus.

Observations on cotton seedlings indicate that parietinus and avenae may attack diseased plant tissues and perhaps utilize as food the contents of cells in early stages of necrosis. In such instances these nemas may conceivably be regarded as saprophytic. Even so the contents of living plant cells constitute part, and probably the greater part, of their food. The importance of these nemas as pathogenic agents is difficult to determine. Either alone or in conjunction with certain fungi they may be of considerable significance in initiating or perpetuating some of those root troubles, the causes of which have been so obscure and perplexing. While the writers' endeavors to determine experimentally the rôle of these and other nemas in the etiology of cotton sore shin have been disappointingly inconclusive, nevertheless neither *Aphelenchoides parietinus* nor *Aphelenchus avenae* can yet be dismissed as merely saprophytic secondary invaders of no significance.

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[3] Christie, J. R., and C. H. Arndt. Further notes on the nematodes associated with the sore shin of cotton. U. S. Dept. Agr., Bur. Plant Indus. Plant Dis. Rpt., 17: 10–12. 1933. [Mimeog.]

OVERWINTERING OF ERWINIA AMYLOVORA IN ASSOCIATION WITH SEVERE WINTER INJURY ON BALDWIN APPLE TREES

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(Accepted for publication Aug. 14, 1935)

The unusually low temperatures of the winter of 1933–34, according to surveys made by R. L. Gillett,[1] Agricultural Statistician of New York (1934), killed outright in New York alone 1,361,000 apple trees of bearing age and left more than 2,000,000 trees crippled. Heavy losses were sustained also by the growers of other fruits, with peaches, sweet cherries, and quinces suffering most.

The presence of winter injury in blight-susceptible orchards altered considerably the winter-pruning program ordinarily consisting of the removal of fire-blight cankers followed by the shaping of the trees. To provide the trees with a maximum of foliage for recovery, pomologists were recommending that pruning operations be suspended until such time during the growing season as would indicate the condition of injury of the trees. This suggestion raised the question of the possible disease hazard from the omission of canker removal. In case the cankers were to be removed it introduced to the grower the difficult problem of distinguishing between canker injury and cold injury.

Accordingly, the effect of winter injury on the incidence of blight was studied in two of the writer's experimental orchards of Bartlett pear and Baldwin apple where there had been severe epiphytotics of fire blight in 1933. The plant-tissue injury in the Bartlett pear orchard was so severe that the bark on many of the trees was blackish, whereas in the Baldwin apple orchard, although considerable sapwood was discolored, the principal injury to the bark was in association with the canker margins. In cases of such diversity it was thought that a bacteriological study of the survival of the fire-blight organism, *Erwinia amylovora*, in the pear and apple cankers in these orchards would help to clarify the situation.

In March, after the last February cold wave, a number of fire-blight cankers from the Bartlett pear orchard of Ralph Nelson at Clyde, N. Y., were brought into the laboratory for study. The injury was so extreme that the discoloration extended throughout the tissues in the majority of the trees examined. This resulted later in the death or severe crippling of a large

[1] N. Y. State Dept. of Agr. and Markets, Bureau of Statistics. Winter damage to fruit trees and grape vines, winter of 1933–34 (Preliminary). In cooperation with U. S. Dept. of Agr., Bureau of Agr. Economics, Div. of Crop and Livestock Estimates. 9 numb. leaves. 1934. [Mimeographed.]

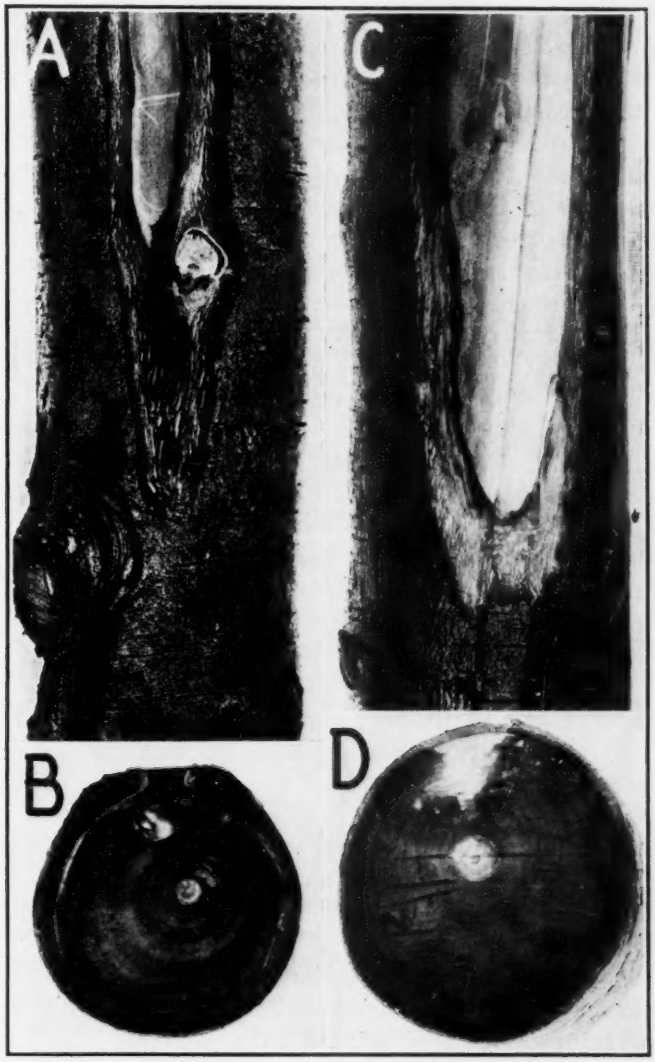

FIG. 1. Winter injury in Bartlett pear. A. Showing severe winter injury to a limb 3 inches in diameter. A cut was made to expose the tissues of the bark, which were blackish and interspersed longitudinally with characteristic light-color streaks. B. Cross section of the limb shown in A. A discontinuous band of light-color tissue in the region of the cambium contained living cells. C. Showing severe winter injury to a blighted limb 3 inches in diameter. The bark from over the canker margin had been cut away to expose the underlying tissues. The characteristic light streaks in black of winter injury are illustrated on the left and on the right is the characteristic reddish brown blighted tissue. D. Cross section of the cankered limb shown above in C. The wood underneath the tip of the canker was apparently normal in color in strong contrast to the dark wood beneath the winter-injured bark.

proportion of the trees. A severely injured pear limb is shown in figure 1, A. The surface of the bark had a blackish cast. When a portion of the bark was cut away the black discoloration was observed to extend to the cambium and throughout the sapwood. In figure 1, B a cross section of the same limb is shown. Only a small layer of tissue located in the region of the cambium appeared alive. Microscopic examination of the cells revealed that the majority of them were discolored, plasmolysed, and apparently dead. In figure 1, C extremely severe winter injury is shown in association with a fire-blight canker. The bark was cut away at the margin to expose the winter-injured and blighted tissue. The winter-injured region on the left shows the characteristic black with many light-color streaks, while the canker tissue on the right shows the usual reddish brown but with fewer light-color streaks. The wood underneath the tip of this canker was almost normal in color (Fig. 1, D) in strong contrast to the dark wood below the winter-injured bark. When cut with a knife the winter-injured tissues in the region of the cambium extruded a watery fluid, indicating that the cells were dead and their membranes had ceased to function.

Attempts to isolate the fire-blight bacteria from 10 cankers on pear gave only negative results. Tissue was plated from 5 different locations around the margin of each canker. Although this study was limited in scope, it appears that the bacteria were unable to survive in cankers under these conditions.

Fire-blight cankers on Baldwin apple trees observed in March showed serious injury of the bark tissues in association with the canker margins. For a variable distance, usually under 1 inch, extending away from the canker margin, the bark tissue was deeply discolored. Moreover, the tissues of the woody cylinder, and particularly of the sapwood, were affected. The discoloration was variable in degree and extent and often ran throughout the bark, being interspersed with numerous light-color streaks typical of winter injury.

Since the winter-injured bark tissues coincided approximately with the usual location of the fire-blight bacteria in dormant cankers,[2,3] it was thought that the cold injury would affect adversely the overwintering of the bacteria and possibly prevent resumption of canker activity in the spring. When the apple bark tissues were observed microscopically some of the discolored cells showed plasmolysis and appeared to be dead, while others appeared to be alive. Still other cells were not even discolored. The fact that not all of the cells were dead precludes the possibility of knowing the effect on the bacteria in apple bark tissue in which all the cells were killed. However,

[2] Jones, D. H. Bacterial blight of apple, pear, and quince trees. Ontario Dept. Agr. Bull. 176. 1909.

[3] Brooks, A. N. Studies of the epidemiology and control of fire-blight of apple. Phytopath. 16: 665–696. 1926.

the case of the Bartlett pear might give some evidence on this point. Except for the already mentioned preliminary isolations from Bartlett pear cankers, all of which were negative, the isolations herein reported were limited to the Baldwin apple trees, all of which survived the winter.

MATERIALS AND METHODS

Isolations were attempted from 345 Baldwin apple cankers from stems ranging between ¼ and 4 inches in diameter. The cankers were removed in March, brought into the laboratory and stored in a 32° F. incubator until the isolations were made. Depending on the size of the limb, 2 to 5 isolations were attempted from each canker. The isolations were made from a band of tissue approximately 1 inch wide outside the canker margin. Several samples were taken from over this range.

The isolation technique consisted of, viz., the disinfestation of the surface of the canker with bichloride of mercury (1–1000), the removal of the exposed outer bark by making cuts back and forth with a sterile scalpel, and the transfer aseptically of cubes of tissue to sterile water in Petri dishes. The cubes were chopped fine with a sterile scalpel and allowed to stand for about 10 minutes before making loop dilutions to 3 successive plates. The plates were poured with nutrient agar and incubated at 24° C. Observations were made at daily intervals for growth and the bacterial colonies appearing were transferred to nutrient agar slopes. A 2-day-old growth on agar was used for pathogenicity tests. Inoculation was by needle puncture into the receptacle cups of flowers of dwarf Bartlett pear forced in the greenhouse. The isolates from different cankers were never inoculated into the flowers of the same cluster. At least one flower in a cluster was left as a check. Observations were made at daily intervals. Positive symptoms or the presence of droplets of ooze usually appeared from 2½ to 4 days after inoculation. The blossoms showing positive symptoms were removed with scissors and stored. Reisolations and reinoculations were made with doubtful cultures.

EXPERIMENTAL

The results of the isolation studies summarized in table 1 provide evidence that the bacteria were able to survive in winter-injured apple tissue. The fire-blight bacteria were recovered from 85 or 24.6 per cent of the 345 cankers examined. From a total of 1061 isolation attempts the organism was recovered 156 times or in 14.7 per cent of the cases. All sizes of cankers yielded the causal organism except cankers on limbs over 3 inches in diameter. In this size group there were but 3 cankers. Of the cankers examined 152, or 44.1 per cent, were apparently sterile.

From the other 193 cankers, in addition to the 156 isolates of the causal bacteria, there were 258 cultures of nonpathogenic bacteria. Of these, 91

TABLE 1.—*Results of attempts to recover the fire-blight organism from dormant cankers on winter-injured Baldwin apple trees*

	Diameter in inches of the cankered limbs					
	¼–½	½–1	1–2	2–3	3–4	Total ¼–4
Cankers examined	6	210	104	22	3	345
Cankers alive	1	51	28	5	0	85
Per cent alive	16.7	24.3	26.9	22.7	0.0	24.6

simulated the fire-blight organism, 26 were white filiform, 33 white spreading, 28 gray to cream color, 50 yellow to orange, and 30 miscellaneous. The exact significance of the secondary invaders has not been determined, but it is possible that at least certain of them may antagonize the fire-blight organism.

The trees from which the cankers were removed during the dormant season were reexamined in the fall of 1934 for evidence of what had happened to the cankers that had escaped the eyes of the operators or that had been left for other reasons (Table 2). The bulk of these cankers had smooth

TABLE 2.—*Results of field examinations in the fall of 1934 for evidences of activity of fire-blight cankers on winter-injured Baldwin apple trees*

	Diameter in inches of the cankered limbs					
	¼–½	½–1	1–2	2–3	3–	Total
Cankers examined	3	5	32	34	42	116
Cankers alive	0	0	15	15	25	55
Per cent alive	0.0	0.0	46.8	44.1	59.5	47.4

margins, a fairly reliable criterion that many of them were alive (Brooks, 1926). A total of 116 cankers were examined for evidence of spread of infection and 55, or 47.4 per cent of them, had apparently moved 1 to 5 inches during the season. This was determined by cutting away the outer bark and examining for winter-injured tissue, a condition that had been quite generally observed in association with the margins of the cankers while dormant. The active cankers showed the usual tissue symptoms beyond the discolored regions. Puncturing the advancing margins with a needle or nail resulted in the issuing forth from the wound of a milky fluid that contained the bacteria in great abundance. Some of these cankers were alive and exudating during the summer of 1935.

DISCUSSION AND CONCLUSIONS

The fact is well known that the fire-blight organism overwinters in a narrow band of tissue outside the canker margin. Although the winter injury to the bark adjoining fire-blight cankers coincided with the usual location of the causal bacteria, it may be concluded from these studies that it does not prevent the bacteria from surviving the winter. Where nearly all the cells were killed, as in the case of Bartlett pear, it was anticipated that the bacteria would die as they ordinarily do in the dead tissue of cankers. In the apple tissue, however, not all of the cells were dead at the time of the examination. The presence of living cells may account for the apparent lack of effect on the bacteria.

A complicating factor, evident at once to those who have studied this disease, is the extreme variability in the percentage of cankers that survive the winter under more normal conditions on the same or different varieties of trees. On the basis of earlier observations on the overwintering of cankers in Baldwin apple trees it appears that a much larger percentage survived in association with the winter injury. It seems that the presence of the bacteria or of the canker tissue increased the susceptibility of the adjacent apparently healthy tissue to cold injury and the cold-injured tissue in turn was apparently more favorable as a medium for the survival of the bacteria. Pomologists are well agreed that any influence that depletes the supply of nutrients to the tissues in fruit trees has a tendency to weaken their resistance to adversity and especially to cold weather conditions.

The decreased vitality of the winter-injured trees seemed to hold the disease in check, which explains the small amount of advance made by the cankers during the subsequent growing season. A further contributing factor was an extremely dry spring season in 1934 during which no oozing cankers were observed in the block of Baldwin trees.

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PHYTOPATHOLOGICAL NOTE

Galls Produced by Plant Hormones, Including a Hormone Extracted from Bacterium tumefaciens.—It has been demonstrated by various workers[1] that plant growth hormones will, in many plants, produce a bending of stems, roots, and petioles, inhibit bud development, speed up callus formation following bud decapitation, and stimulate root production in cuttings. The work reported in this paper adds to the above list the production of galls.

The growth substances used for gall production were indoleacetic[2] and indoleproprionic acids. They were used in a salve in which 20 milligrams of the growth substance were mixed with one gram of lanolin. The lanolin mixtures were applied to decapitated stems and side wounds on stems as a film smear, completely covering the wound. By another method, stems were smeared without wounding.

The experimental plants were beans, tobacco, tomato, sunflower, privet, Paris daisy, *Bryophyllum pinnatum, Kalanchoe daigremontiana* and *Impatiens balsamina.*

Galls were produced by smearing the wounded stems with a film of the mixture. Only one application was necessary. (Figs. 1 and 2).[3] It might be stated here that in the production of bacterial and fungus galls, occurring either naturally or by artificial inoculation, a lesion is necessary for the entrance of the organism. Bending of stem or petiole may occur in a few hours by the mere application of the growth substance to the uninjured epidermis, but it appears that a definite wound is necessary for gall production. Bean, tobacco, and sunflower plants were the most satisfactory used in these experiments for gall production, although Paris daisy, privet, and *Impatiens balsamina* also produced definite galls. Bryophyllum and Kalanchoe responded by forming a few roots. Ricinus, though tried repeatedly, showed no trace of root or gall formation. Tobacco (Fig. 2, B) and sunflower produced galls rather slowly. None of the hormone galls developed so rapidly as did those of a *Bacterium tumefaciens* gall growing on a favorable host. However, in the case of the bean, the galls produced by a very virulent culture of *Bacterium tumefaciens* (Fig. 4, B and C) never reached the size of the galls produced by indoleacetic acid on bean (Fig. 1).

[1] No attempt will be made in this brief note to enumerate the workers on plant hormones. For a starting point on the literature of this subject, readers are referred to a fairly recent summary by F. W. Went in Botanical Review 1: 162–182. 1935.

[2] The indoleacetic acid was obtained through the courtesy of Dr. F. W. Went of the California Institute of Technology, and Dr. N. L. Drake of the University of Maryland. It can now be obtained from reputable chemical firms.

[3] One-half the cost of publishing the illustrations accompanying this paper was borne by the authors.

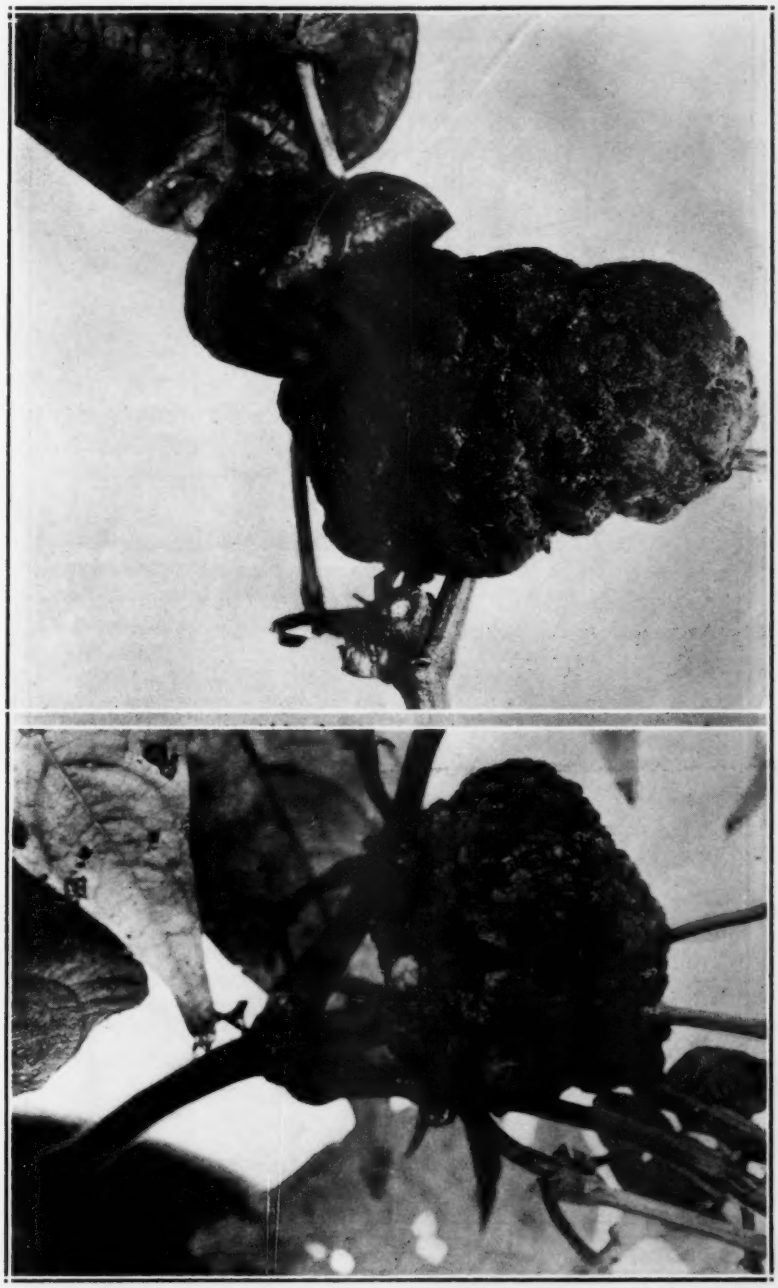

FIG. 1. Galls, produced on Red Kidney bean plants by decapitating and smearing the wound with indoleacetic acid (hetero-auxin) November 18, 1935. Photographed April 25, 1936, while galls were still growing. A little less than natural size.

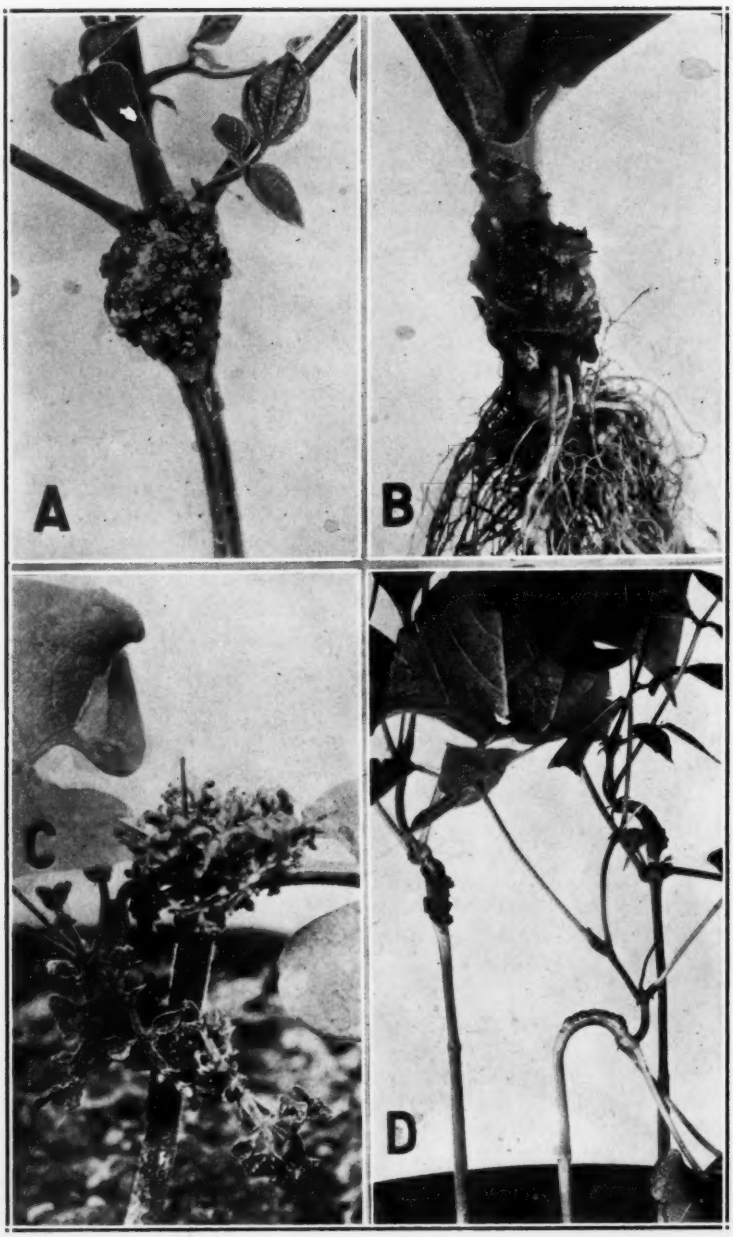

FIG. 2. A. Red Kidney bean seedling wounded at side and smeared with indoleacetic acid February 6, 1936. Photographed April 1, 1936. B. Tobacco seedling wounded and smeared with indoleacetic acid February 21, 1936. Photographed April 14, 1936. C. Very young Red Kidney bean seedling decapitated and smeared with indolepropionic acid February 10, 1936; witches'-broom produced. D. Three Red Kidney bean seedlings smeared on stem with indolepropionic acid January 6, 1936. Photographed February 2, 1936. A–C, a little less than natural size; D, about $\frac{1}{2}$ natural size.

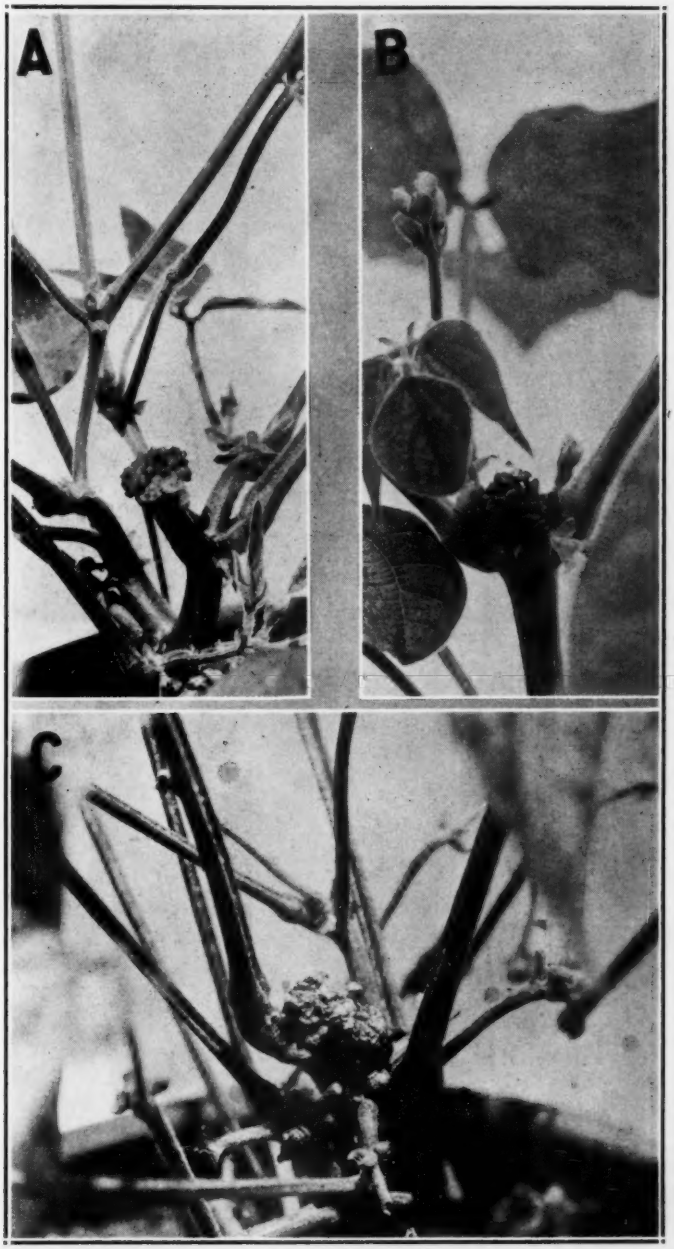

FIG. 3. A. Gall on Red Kidney bean seedling produced by decapitating and smearing with growth substance extracted from sterilized *Bacterium tumefaciens* cultures March 5, 1936. Photographed April 1, 1936. B. Decapitated bean seedling smeared with indole-acetic acid and photographed at the same age as A. C. Another gall on a bean seedling produced by decapitating and smearing with growth substance extracted from sterilized *Bact. tumefaciens* cultures March 5, 1936. Photographed May 22, 1936. A little less than natural size.

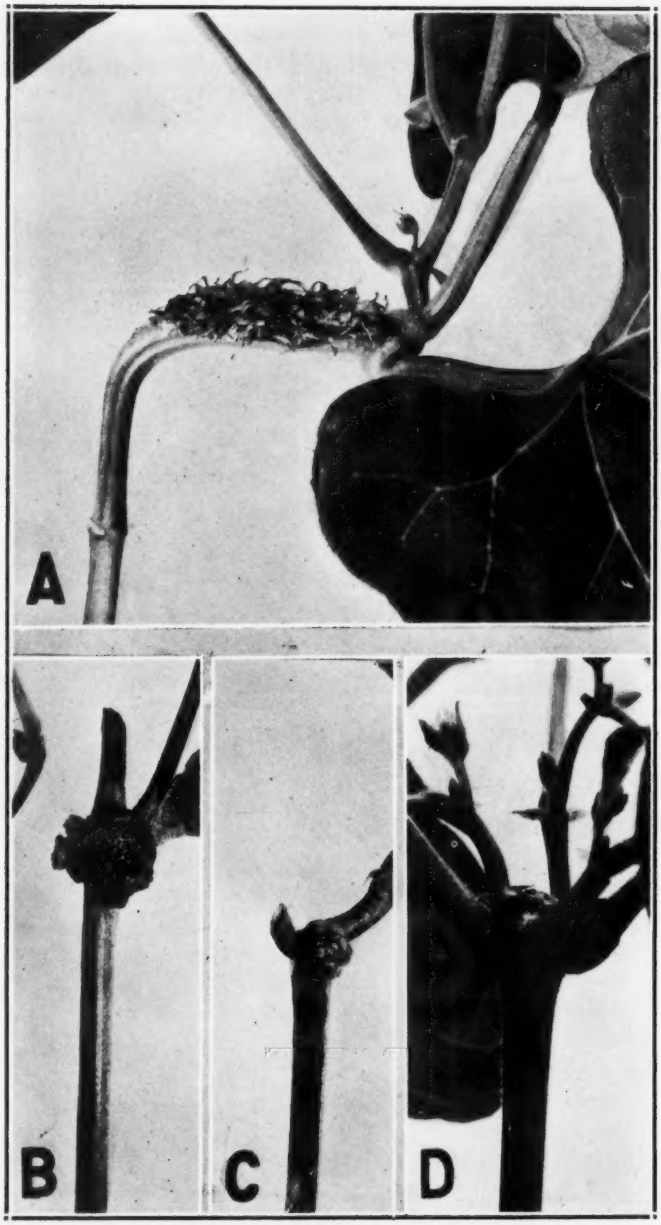

FIG. 4. A. Red Kidney bean seedling smeared with indolepropionic acid January 6, 1936. Photographed February 6, 1936. B. and C. Red Kidney bean seedlings inoculated with *Bacterium tumefaciens*. B. By puncturing. C. By decapitating December 16, 1935. Photographed April 25, 1936. D. Red Kidney bean seedling decapitated and smeared with lanolin only for control. Same age as those in figure 3, A and B. Natural size.

Tomato and bean stems produced bending and root primordia by surface smearing without wounding. (Fig. 2, D and 4, A). Galls with root rudiments and witches'-brooms (Fig. 2, C) were produced on bean plants by decapitating the $\frac{1}{4}$ to $\frac{1}{2}$ in. growth of stem immediately above the first two primary leaves and smearing the wound with indolepropionic acid. Attention might be called here to the fact that witches'-brooms have been produced on tomato and geranium plants by inoculating decapitated stems with *Bacterium tumefaciens* cultures.

Galls produced with a growth substance extracted from Bacterium tumefaciens.—In a theory of gall formation it is not the mere presence of the organism that leads to overgrowths but rather the stimulus of certain products of the organism's metabolism. The preceding work suggested the possibility of producing galls with a growth substance derived from cultures of *Bacterium tumefaciens.*

To this end, the organism was grown in a synthetic medium containing 2 per cent dextrose, 1 per cent peptone (Difco) and a small amount of tryptophane, in addition to the usual inorganic salts, and subsequently extracted with peroxide-free ethyl ether. Upon evaporation of the ether, the residue was taken up, without further purification, in 0.5 g. of pure lanolin.

This lanolin mixture of extracted growth substance was applied to the plants in the manner already described. On bean seedlings (Fig. 3, A and C), and on daisy stems, galls have thus been produced that grow at about the same rate as those produced by indoleacetic acid (Fig. 3, B).

The pure lanolin controls in these experiments have been negative on the various plants (Fig. 4, D). In a few cases nodular growths started on bean plants at the place of lanolin application, but did not continue development beyond a few millimeters.

No definite information relative to the chemical nature of the growth-promoting substance from *Bacterium tumefaciens* or to the best method of its extraction has yet been obtained. Work with these extractions is however, being continued.

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